Examination of the urine and other clinical side-room methods (late Husband's) / by Andrew Fergus Hewat.

The Examination of the Urine

by A. Fergus Hewat

DIRE(

TO TEST URINE FOR SUGAR.

Pour about five cc (one large teaspo
tube, and add eight drops (not more) of urin
bubbling boiling water and leave it there, wi
Remove from the water and allow to stand
remains clear the urine is free from sugar ; if
of removing the tube from the water, there
bulky green precipitate present when the tub
sugar (0.1 to 0.5%) ; a dense yellow precipit
while a thick red precipitate proves that there
A faint white or blue turbidity, forming as th

ONS.

l) of the blue Benedict solution into test-
Mix by gently shaking. Place the tube in
ne water actually boiling, for five minutes.
isturbed for two minutes. If the solution
reenish turbidity forms within two minutes
a trace of sugar (under 0.1%); a more
aken from the boiling water indicates more
hows a still larger amount (0.5 to 2.0%);
very large amount of sugar (over 2.0%).
ution cools, is of no significance, especially

Examination of the Urine

and other

Clinical Side-Room Methods

(late Husband's)

By

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FIFTH EDITION

EDINBURGH:
E. & S. LIVINGSTONE, 15-17 TEVIOT PLACE
1914

PREFACE

THE methods of examining urine, blood, stomach contents, sputum, etc., are generally taught in practical classes during the first three months of the course in clinical medicine.

The following pages are written to give the student a short description of these methods. It is hoped by this means that he will be saved much time in note-taking and searching through larger text books.

The first part of the book dealing with the urine is the result of a very complete revision, amounting to a re-writing of the fourth edition of Husband's book on "The Urine in Health and Disease." During the preparation of the book, the latest editions of the standard text books on clinical diagnostic methods have been consulted. The author acknowledges particularly the assistance of Emerson's

"Clinical Methods," third edition, "Panton's "Clinical Pathology," Morris' "Clinical Laboratory Methods," and Dixon Mann's "Physiology and Pathology of the Urine," and Gulland and Goodall's book on "The Blood." The author has great pleasure in expressing his indebtedness to Dr John D. Comrie for much valuable help in the preparation of the book and in proof reading. He is also indebted to Mr John Fraser for several valuable suggestions.

It is a special pleasure to acknowledge the kindness and help of the publishers in seeing the book through the press.

A. FERGUS HEWAT.

3 Darnaway Street
Edinburgh, *June 1914*

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THE URINE

COLLECTION OF URINE

The examination of the urine as a routine procedure is apt to be neglected in general practice. It cannot be too strongly urged that a thorough, systematic examination should be made with much greater frequency than is at present usual.

In dealing with most cases it is necessary to get a sample from the urine passed during the previous twenty-four hours. In hospital work this is easily accomplished. The urine is measured, and a sample set out during the small hours of the morning. In private work directions must be given as to the preservation of all urine passed, and a sample for examination is taken from the total amount passed.

It is convenient to start the day's collection when the patient first voids urine in the morning, *e.g.*, 8 a.m. The quantity passed at this time is discarded, and all urine passed during the next twenty-four hours, *i.e.*, up to and including the urine passed at 8 a.m. next day, is collected for measurement and removal of the daily sample.

At times it is necessary to separate the urine passed during the day from that passed at night. The day may count from 6 or 8 a.m. to 9 or 10 p.m. In some cases the urine voided at certain times requires to be tested without mixing it with the rest of the urine passed.

It is frequently necessary to put some preservative in the specimen of urine to prevent bacterial decomposition. If the specimen of urine is to be submitted to chemical examination, the specimen bottle may be rinsed out with chloroform and a few drops left in it. This preservative is convenient as it can be easily driven off, but the bottle must be tightly corked to prevent loss of chloroform by volatilization. Crystals of thymol may also be used as a preservative.

The sediment of urine may be obtained by allowing a specimen to stand in a tall glass for a few hours. If the sediment be required more quickly, and in a more concentrated form, a sample of the urine must be centrifuged. This can be done by a hand or electric centrifuge.

QUANTITY OF URINE

In Health the average amount of urine

passed in twenty-four hours is about 40-50 ounces (1100cc–1400cc). This varies considerably, and the quantity passed is largely influenced by the amount of fluid and nitrogenous material ingested. The action of the skin also has an important bearing on the amount of urine passed. In hot weather, less urine is passed than in cold weather owing to the much freer action of the skin in summer than in winter. A healthy kidney excretes more urine during the day than during the night. Any severe exertion will diminish the flow of urine, because more fluid is lost by the lungs and skin than is normal.

In Disease the amount of urine varies considerably.

An increased output is seen in diabetes mellitus and insipidus, hysteria, chronic interstitial nephritis, amyloid disease of the kidney, renal tuberculosis, and in cases of hypertrophy of the left ventricle of the heart. It must also be remembered that more urine is excreted at night compared with the amount passed during the day by patients suffering from heart and kidney disease. This fact shows a definite difference between a functional and an organic polyuria.

A diminished output of urine is present in patients suffering from any febrile condition, diarrhœa, acute and certain types of chronic nephritis and chronic heart disease with broken-down compensation.

It must also be kept in mind that a deficient output of urine may be due to retention of urine in the bladder brought about by a stricture of the urethra or enlarged prostate.

CERTAIN DRUGS affect the quantity of the urine. Cardiac tonics, such as caffein, digitalis, etc., cause an increased output. Drugs acting on the kidney itself cause an increased flow, *e.g.*, pot. acetate, pot. citrate, etc.

SPECIFIC GRAVITY OF THE URINE

The instrument used for estimating the specific gravity of urine is known as an urinometer. It is advisable to get a thoroughly good instrument, because much valuable information can be obtained from a correct reading of the specific gravity.

The specific gravity means the weight of a certain volume of urine compared with the same volume of distilled water. An estimate is, therefore, obtained of the quantity

of dissolved salts, etc. which are present in the urine.

In estimating the specific gravity certain precautions must be taken, viz. :—

1. The urine should be cold, especially for Life Assurance cases. For example, a specific gravity of 1012 may become 1016 on cooling ;
2. The instrument must be accurate ;
3. The urinometer must float freely in the urine, and not touch the sides or bottom of the specimen glass ;
4. In reading the scale the eye must be opposite the lower level of the meniscus.

In Health the specific gravity of urine varies between 1015-1025. The specific gravity is diminished by taking large draughts of water on an empty stomach. The specific gravity is raised after a long fast, or a full meat diet, after exercise, or long abstinence from liquid.

In Disease the specific gravity is *increased* in diabetes mellitus due to the sugar dissolved in the urine, in all febrile urines due to the high concentration of solids, in acute nephritis, in diarrhœa, and in wasting diseases accompanied with much sweating.

The specific gravity is *diminished* in cases

suffering from any cachetic state, in some cases of chlorosis, diabetes insipidus, nervous states, hysteria, cirrhosis of the kidney, etc.

The most important substance dissolved in normal urine is urea, and next in importance are the chlorides. These two substances constitute the largest quantity of dissolved solids in the normal urine.

A rough estimate of the dissolved solids may be obtained by multiplying the last two figures of the specific gravity by 2·3. The result obtained gives the number of grammes of solids per litre of urine.

e.g., sp. gr. $1022 \times 2{\cdot}3 = 50{\cdot}6$ grms. per litre,
or, 5·06 per cent. solids.

ODOUR

In Health the odour of urine is characteristic. If it is fairly concentrated, or ammoniacal decomposition is taking place, the intensity of the odour is increased.

In Disease certain characteristic odours are noticeable. In cases of cystitis the urine has a fishy smell, and when well-marked cystitis is present, the urine develops an ammoniacal smell due to decomposition brought about by the presence of organisms. A saccharine urine frequently gives a

sweetish odour like new-mown hay, due to the presence of acetone. In cancer of the bladder the odour of the urine is offensive and intense in character.

Abnormal urinary odours may be due to drugs or diet. Certain substances, ingested as food or drugs, give peculiar and distinctive odours to the urine, *e.g.*, turpentine gives the urine a smell of violets.

The balsams, copaiba, and cubebs all give an abnormal odour to the urine. Shortly after eating asparagus we note the characteristic smell in the voided urine.

N.B.—An abnormal urinary odour may be due to the bottle in which the specimen is brought.

COLOUR

The colour of urine is due to several pigments, chief amongst which is urochrome, which give to urine the normal yellow colour. Other pigments are also present such as :—

(a) Hæmatoporphyrin, in small amounts ;

(b) Uroërythrin in certain cases. This pigment gives the salmon-red colour seen in a deposit of urates ;

(c) Urobilin develops from its chromogen urobilinogen after the urine

is voided and may be termed a normal constituent.

Other pigments which occur in urine, such as bile, blood, etc., are strictly pathological.

The colour of urine is best estimated by examining it in a "specimen glass" by direct and transmitted light. Normally, we find the colour to be some shade of yellow. With a view to a definite nomenclature a colour scale has been prepared, and is known as Vogel's colour scale. The colours on this scale can be compared with the urine. A definite description is attached to each colour on the scale.

Acid urine tends to be darker than alkaline urine.

Urine is usually transparent, but may be rendered translucent by the suspension of mucus, epithelium, salts, urates, pus, bacteria, or blood. When small quantities of blood are present the urine is darker, and in such cases is termed "smoky."

The causes of abnormal colouration in the urine will be found in the accompanying table :—

Colour.	*Cause.*
Pale or Colourless	Hysteria, diabetes, cirrhotic kidney, anæmia, debility.

Colour.	*Cause.*
Dark Yellow to Brown	Fevers, or the febrile state.
Orange	Santonin, or drugs excreted as chrysophanic acid, *e.g.*, rhubarb, senna, etc. If rendered alkaline it becomes reddish in presence of these drugs.
Red	Blood (when amount is large), eosin derived from certain sweets, phenolphthalein if the urine be alkaline.
Smoky	Small quantity of blood in acid urine.
Greenish to Brown	Bile, salol, resorcin, etc.
Brownish to Black	Carbolic acid, melanin, alcaptonuria. Sometimes this colour is given in phthisical patients and in cases of hæmoglobinuria and hæmatoporphyrinuria.
Greenish to Blue	Indigo products in putrefying urine, methylene blue.
Greenish-Yellow	Diabetic urines at times have this colour.
White or Opaque	Chyle, pus, or globulin.

REACTION

This is determined by red and blue litmus papers. Blue litmus papers should be kept in a carefully-stoppered bottle, because the

acid in the air from other re-agents will turn the blue litmus red and make it useless. An acid turns blue litmus red, and an alkali turns red to blue.

In Health urine is distinctly acid, due to the presence of acid phosphate of soda (NaH_2Po_4). The acidity rises at night and during fasting. During digestion the acidity sinks, and may even give place to alkalinity from the presence of basic phosphates. This change is known as the alkaline tide, and is most marked about three to four hours after a meal, especially a heavy dinner.

If a specimen of freshly voided urine turns red litmus blue the alkalinity may be due to (1) a fixed alkali (*e.g.*, potassium), or (2) a volatile alkali (ammonia). To determine which factor causes the alkalinity, the litmus paper which has been turned blue is allowed to dry. If the blue colour remains after drying, the reaction is due to a fixed alkali, if the red returns, it is due to a volatile alkali.

It is frequently important to determine this point, because an alkalinity due to a fixed alkali reflects a condition of the blood, and an alkalinity due to ammonia indicates

an inflammatory condition of the urinary tract, especially in the bladder.

The intensity of the reaction of the urine varies much with diet. Herbiverous animals, for example, pass alkaline urine, but when they are starved their urine becomes acid, due to the tissue proteid being used up. The urine of vegetarians is less acid than normal, it may even be alkaline in its reaction. The greater the amount of proteid oxidised in the individual the greater will be the intensity of the acid reaction.

In Disease acidity is increased in fevers, especially acute rheumatism, in acid dyspepsia, gout, and chronic rheumatism and diabetes mellitus. In the last case the change is due to organic acids (*e.g.*, oxybutyric, etc.), and the same occurs also in that rather obscure condition known as "acidosis." The acidity of urine is temporarily increased by active exercise, by the administration of mineral acids, and by benzoic acid.

Highly acid urines tend to deposit urates and uric acid with a liability to the formation of calculi.

Diminished Acidity occurs after cold bathing, and the taking of alkaline medicines or vegetable acids. In cystitis, anæmia, and great debility from many causes, the

urine is often alkaline. *Alkaline urine* is frequently passed when there is any interference with the emptying of the bladder, and may therefore occur with an enlarged prostate, myelitis, stricture of the urethra, etc. This type of alkaline urine is invariably brought about by the presence of the micrococcus ureæ which decomposes the urea, forming ammonium carbonate. In alkaline urines there is very commonly a deposit of phosphates with a tendency to the formation of phosphatic calculi.

TOTAL ACIDITY OF THE URINE.—This may be estimated in a relative way by titration against phenolphthalein. Only approximate results can be obtained due to the difficulty in determining the end reaction, but each observer should determine the end reaction for himself, and this will give a satisfactory comparative result for different urines in determining the titration acidity.

METHOD.—The titration acidity is estimated by Folin's method, viz., 25cc. of urine are placed in an Erlenmeyer flask. Add to this 15 grms. of neutral potassium oxalate to precipitate the calcium salts which, if left in solution, would interfere with the sharpness of the end-reaction. A drop or two of 1% phenolphthalein solution is next

added and the whole mixed thoroughly for 2-3 minutes. From a burette add $\frac{N}{10}$ sodium hydroxide till a permanent faint pink colour is produced in the urine.

CALCULATION.—Express in terms of $\frac{N}{10}$ soda. Thus 6cc. of $\frac{N}{10}$ are required to neutralise 25cc. urine, therefore $6 \times 4 = 24$cc. $\frac{N}{10}$ soda are equal to 100cc. urine, *i.e.*, 24cc. $\frac{N}{10}$ NaOH are required to neutralise 100cc. urine.

NITROGENOUS SUBSTANCES

The total urinary nitrogen output varies much according to the type of diet taken. We find also that proteid metabolism can best be estimated by calculating the nitrogenous output in the urine.

It is, therefore, impossible to lay down any hard and fast figures for the relative quantities of the nitrogenous output, because it depends to a very large extent on the diet. Folin gives two tables which indicate a rough average of the relative amount of the nitrogenous constituents on different types of diet :—

TABLE I—Rich Nitrogenous Diet.

TABLE II—Low Nitrogenous Diet.

I

Total Nitrogen . .	14·8 – 18·2 grms.
Urea-Nitrogen . . .	86·3 – 89·4%
Ammonia-Nitrogen . .	3·3 – 5·1%
Creatinin-Nitrogen . .	3·2 – 4·5%
Uric-Acid-Nitrogen . .	0·5 – 1·0%
Undetermined Nitrogen .	2·7 – 5·3%

II

Total Nitrogen . .	4·8 – 8·0 grms.
Urea-Nitrogen . .	62·0 – 80·4%
Ammonia-Nitrogen . .	4·2 – 11·7%
Creatinin-Nitrogen . .	5·5 – 11·1%
Uric-Acid-Nitrogen . .	1·2 – 2·4%
Undetermined Nitrogen .	4·8 – 14·6%

TOTAL NITROGEN ESTIMATION.—The most satisfactory method of estimating the total nitrogen output is by using Kjeldahl's method, which is not applicable to strictly 'side-room' work, because it requires too much time and accurate knowledge with special apparatus.

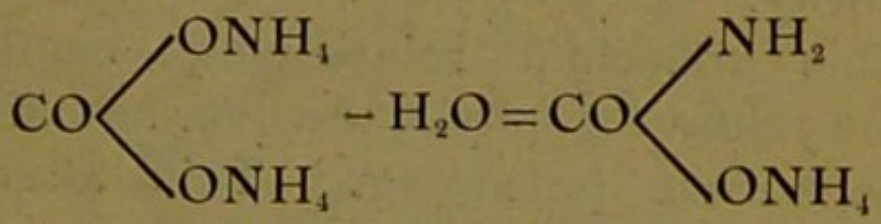
A record of the total nitrogen output is not often required in ordinary clinical work, unless metabolism experiments are being carried out.

UREA ($CO(NH_2)_2$)

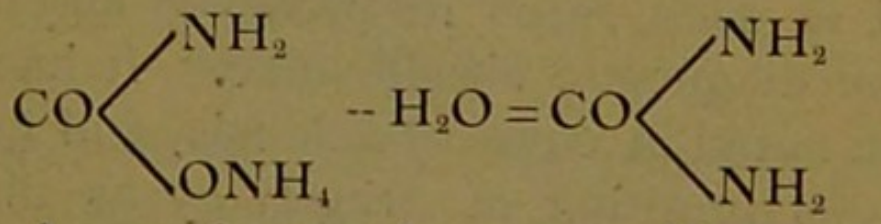
Urea is the chief nitrogenous body in the urine. The average amount excreted in

twenty-four hours by an average individual on an ordinary diet varies from 300-600 grns. (20-40 grms.)

There is some doubt as to where exactly urea is formed in the body, but it is known that proteid metabolism takes place largely in the muscles. This results in the formation of ammonia and sarcolactic acid, and possibly ammonium lactate is formed. The tissues convert this into a carbonate. Dehydration is brought about by the liver cells, and ammonium carbonate is changed to ammonium carbamate, and finally to urea. This is illustrated by Dixon Mann thus :—



Ammonium carbonate.



Ammonium carbamate. Urea.

Urea is present in the urine to the extent of 2 to 3 per cent., ·03 to ·1 per cent. in blood and ·05 to ·1 per cent. in sweat.

Excretion of urea is increased during the acute stage of fevers, acute inflammatory

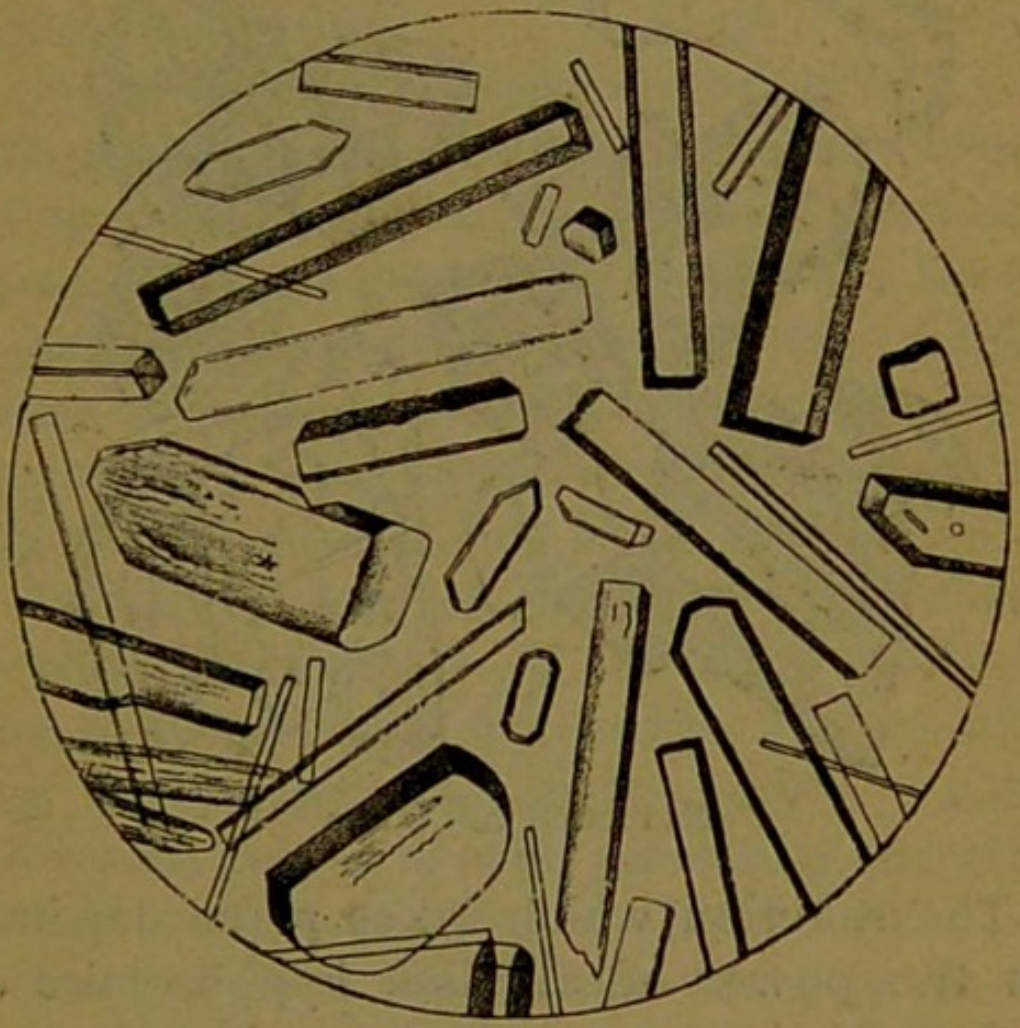
diseases, acute wasting diseases, in the first few days after childbirth, and in diabetes mellitus where the excess may be three or four times above the normal.

EXCRETION OF UREA IS DIMINISHED in chronic cases, when the intake of nitrogenous elements is diminished, and after the acute state of a fever is over. It is also diminished in all types of nephritis—this constituting a very characteristic sign of a recent or old-standing inflammation of the kidney. The amount of urea output may be diminished while the ammonia output rises, thus keeping the total nitrogen at its normal quantity.

This abnormal relationship occurs in acute yellow atrophy of the liver and in acute phosphorous poisoning. This alteration has also been noted in cases of starvation, and in such cases its presence may be of some diagnostic significance. It has also been noted in cases of acidosis, and in pernicious vomiting of pregnancy as distinct from reflex vomiting.

TEST FOR UREA.—A small quantity of urine is evaporated down to one-fourth of its bulk. To this are added a few drops of strong nitric acid. After a few seconds crystals begin to form, and these, when

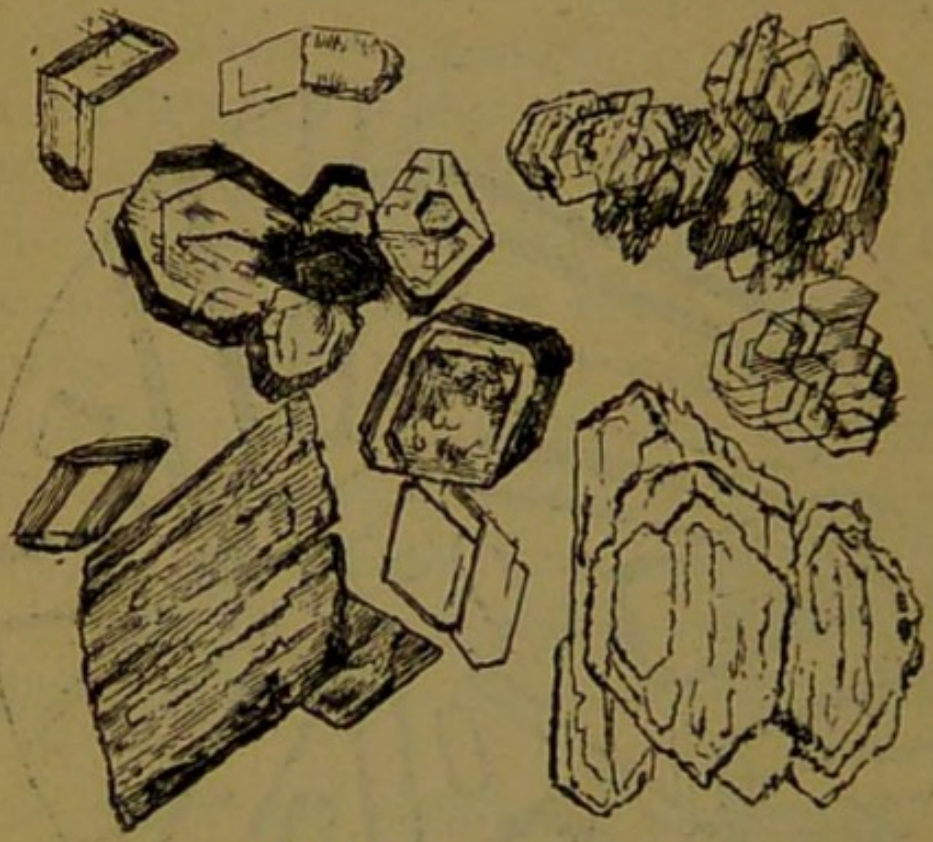
examined by the microscope, are found to consist of rhombic plates of nitrate of urea.



Urea Crystals

Estimation of Urea.—Several methods of estimating urea are in vogue. Most of them are rather too complicated for 'side-room' work. The most convenient method, and that still generally used in clinical work, is known as the sodium hypobromite method. It is freely condemned by chemical physiologists as being too inaccurate, but for

clinical guidance it forms a good enough indication for treatment.



NITRATE OF UREA

The reaction which takes place depends on the power of alkaline hypobromite of soda to decompose urea into nitrogen and carbon dioxide.

$$CO(NH_2)_2 + 3NaBrO = N_2 + 3NaBr + 2H_2O + CO_2.$$

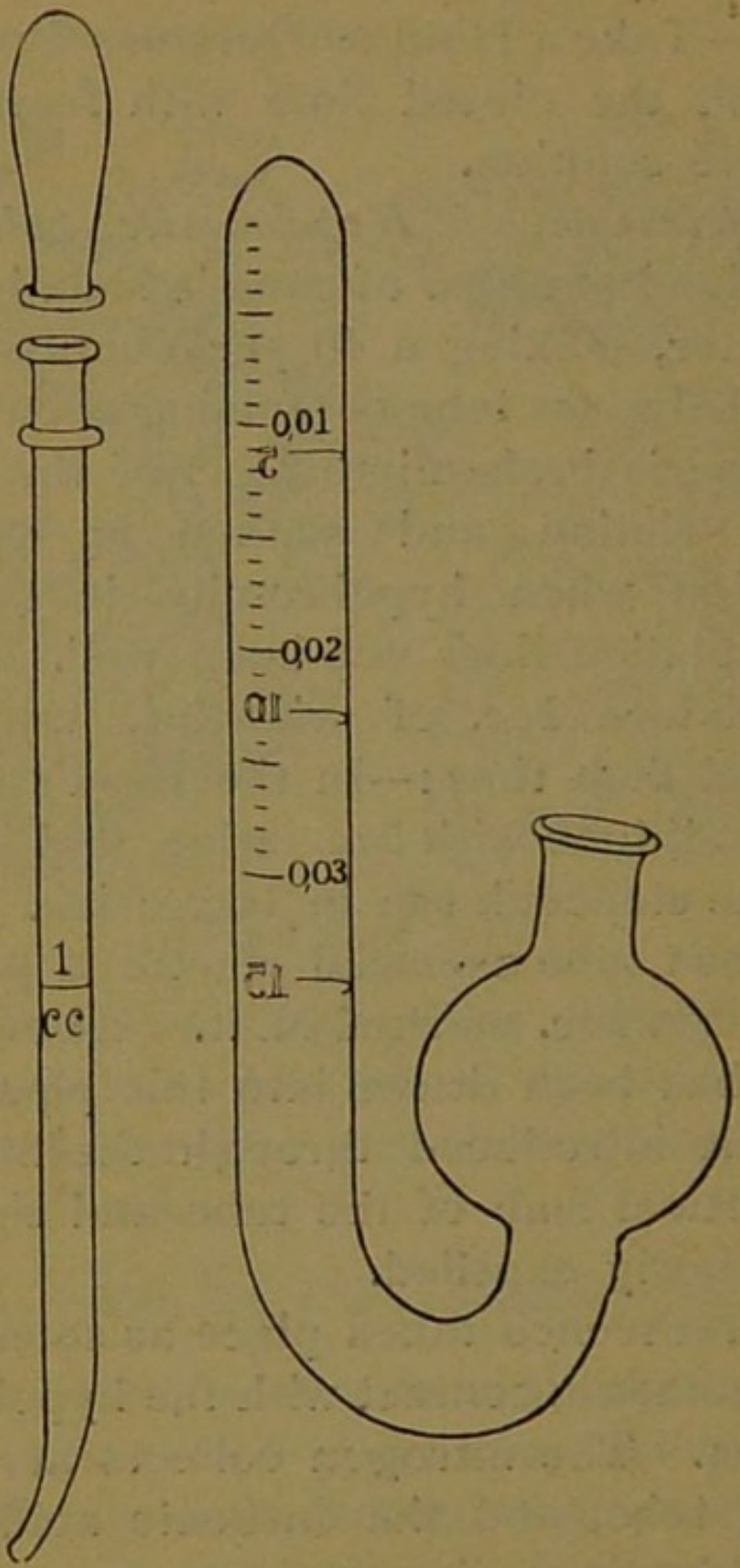
This process measures not only the urea, but also some of the purin bodies which are broken up as well in the chemical change, and there is no correction for vapour and atmospheric pressure, but these go largely to neutralise each other.

METHOD OF CARRYING OUT THE ESTIMATION.—Take a Hind or Doremus Ureometer and fill the closed limb with fresh hypobromite solution.

Preparation of Hypobromite Solution.—Dissolve 100 grms. of caustic soda in 250cc. of water, making a 40 per cent. solution. A sealed glass tube containing about 2cc. of bromine is broken into 25cc. of the caustic soda solution and shaken up on each occasion when hypobromite is required. The solution does not keep well.

Introduce 1cc. of urine into the closed vertical limb thus:—In the Hind's tube fill the small tube with 5cc. urine, and by turning the stopcock run in 1cc. urine. In the Doremus tube a special pipette is provided which has 1cc. marked on it. When 1cc. of urine has been drawn into this pipette, the point is introduced through the bulb into the vertical limb of the tube and the urine very slowly expelled.

Effervescence takes place as soon as the urine comes in contact with the hypobromite solution. The nitrogen collects at the top of the tube, and the carbonic acid is absorbed by the sodium hydrate present. The tube is put aside for about twenty minutes till the froth subsides and then the reading



DOREMUS UREOMETER
(with special pipette)

is taken. Most tubes have a scale of centigrammes of urea per cc. on one side, and grains per ounce on the other side. To obtain a percentage reading the centi grammes per 1cc. are multiplied by 100. To get grains per ounce from the above reading one must multiply by 4·375.

Example :—Tube reading—·03, *i.e.*, 3 per cent. of urea, or $3 \times 4{\cdot}375 = 13{\cdot}125$ grains per ounce.

N.B. It must be remembered that no reading of urea can be taken by itself without estimating the quantity of urine passed in the 24 hours. The total average amount of urea passed in 24 hours is about 450 grains, which, with a normal amount of urine (50 ounces), means about 9 grains per ounce.

AMMONIA NITROGEN

A normal adult on an average diet is said by some authorities to excrete from ·3 to 1·2 grms. of ammonia daily. By others this reading is considered too high.

In health the amount of nitrogen passed as ammonia amounts to 4 per cent. or 5 per cent. of the total nitrogen passed, provided the patient is taking an ordinary mixed diet.

This is frequently spoken of as the ammonia nitrogen.

The out-put of ammonia is *increased* by the ingestion of organic or inorganic acids in the body—the ammonia here being increased as a defensive procedure to conserve the alkalinity of the tissues against the inroads of the acidosis, in oxygen-starvation, diabetes mellitus, where marked acidosis develops, and in certain cases of cirrhosis of the liver, due to the failure on the part of the liver cells to produce urea. Finally, it is to be noted that the ammonia out-put is increased in many cases of toxæmic vomiting of pregnancy, even as high as 20 to 40 per cent. of the total nitrogen. This may be of considerable value in differentiating this type of vomiting from a true reflex vomiting of pregnancy.

ESTIMATION OF AMMONIA NITROGEN.—This may be done in one of several ways, *e.g.*, Folin's, Schlösing's, or the Formaldehyde Method.

FOLIN'S METHOD requires a special apparatus. Schlösing's is the method most frequently used. It takes some time to do, and the readings obtained are rather high.

SCHLÖSING'S METHOD.—25cc. of urine are

mixed with 10cc. of milk of lime. This mixture is placed in a broad flat vessel, *e.g.*, a large Petri dish, a triangular stand is laid on this flat dish and over it is placed a smaller vessel containing 20cc of $\frac{N}{10}$ H_2SO_4. A bell jar is placed over the whole, and the foot of the jar is well greased to render it air-tight. This preparation is put aside for 3 to 4 days. During this time the ammonia in the urine is set free by the milk of lime, which does not affect the urea, and is absorbed into the sulphuric acid. The acid is now titrated against $\frac{N}{10}$ NaOH, and the number of ccs. of $\frac{N}{10}$ NaOH used, multiplied by 1·7, gives the weight in milligrammes of ammonia present in 25cc. urine.

FORMALDEHYDE METHOD.—This method is described as a conveniently rapid method of estimating the ammonia present. The reading obtained is rather high because the amido-acids react in a similar way. However, for quick clinical work the method is quite satisfactory and sufficiently accurate.

This method depends on a combination taking place between ammonium salts and formaldehyde to form hexamethylene-tetramine (urotropin). At the same time the acids combined with the ammonium salts are

liberated, and can be titrated with standard alkali.

$$4NH_4Cl + \text{Formaldehyde}\ 6CH_2O =$$
$$N_4(CH_2)_6\ \text{Urotropin} + 2H_2O + 4HCl$$

METHOD OF PROCEDURE.—Proceed as on page 12 to estimate the acidity in terms of $\frac{N}{10}$ NaOH. When the urine has been completely neutralised, add 10cc. of neutral formaldehyde solution, which is prepared by diluting 5cc. of commercial formalin (40%) with 5cc. of water, and neutralising this solution with $\frac{N}{10}$=NaOH against phenolphthalein.

Fill a burette with $\frac{N}{10}$ NaOH. Read off the level of the fluid, and then add the alkali drop by drop to the urine until a pink colour develops and persists. Again read off the burette and find the quantity of $\frac{N}{10}$ NaOH used.

EXAMPLE OF CALCULATION :—

1. Acidity of urine to phenolphthalein—

3·2cc. $\frac{N}{10}$ NaOH neutralise 25cc. urine,
i.e., 12·8cc. $\frac{N}{10}$ NaOH " 100 " "

2. Total Ammonia—

6·4cc. $\frac{N}{10}$ NaOH require to be added to neutralise the acid liberated by the addition of the formalin.

1cc. $\frac{N}{10}$ NaOH = ·0017 grammes ammonia.

∴ 6·4 × ·0017 = ·0109 grms. NH_3 in 25cc. urine

= ·0436 " " 100 cc. "

The amount passed per diem can be estimated from this.

e.g., 1500 cc. urine per diem

·0436 × 15 = ·65 grms. NH_3 per diem.

The amount of ammonia nitrogen excreted can easily be estimated by remembering that 17 grms. NH_3 contain 14 grms. N.

Therefore ·65 grms. × $\frac{14}{17}$ = ·54 grms. N excreted as ammonia-nitrogen.

If the total nitrogen excreted per diem is known, then the percentage of ammonia-N can be estimated thus— $\frac{\cdot 54 \times 100}{x}$

x = total nitrogen excreted per diem.

3. A somewhat similar method may be employed in calculating the quantity of urea and urea-nitrogen passed per diem.

e.g., By the ureometer scale it is seen that 1cc. of urine contains ·02 grms. urea. If 1500cc. be the daily output of urine, then the quantity of urea passed per diem will be ·02 × 1500 = 30 grms. The amount of nitrogen voided as urea may be estimated by remembering that 60

grms. urea contain 28 grms. nitrogen. Therefore the urea-nitrogen in this case would be $30 \times \frac{28}{60} = 14$ grms. urea-nitrogen per diem.

Normally, a patient passes about twenty times more urea-nitrogen than ammonia nitrogen. It must be distinctly understood that the above method of urea-nitrogen estimation is a rough one.

If the total nitrogen excreted in the day be known, the percentage of urea-nitrogen can be estimated.

URIC ACID

Uric acid occurs in the urine generally combined with alkalies to form normal urates, acid urates, or bi-urates. It may be found free in the urine, and being nearly insoluble (1 in 15,000 of plain water) forms a crystaline deposit. The urates may separate out as crystals, and if they do so in the urinary passages gravel willform. Normally, about 7-10 grains (·7 grms.) of uric acid are excreted daily by a normal individual in health.

An increase of uric acid is to be found in patients suffering from certain diseases, viz., fevers, in functional and organic diseases of the liver, after an attack of gout (although

diminished during the paroxysm), splenic diseases, leucocythæmia, and in some lung diseases where there is a deficient elimination of CO_2.

The output of uric acid is diminished or absent in acute yellow atrophy of the liver, also in chronic gout. It is of importance to distinguish between an absolute increase of uric acid in the urine and an increased tendency to form a precipitate. The amount of sedimented uric acid is increased by (1) the presence of a large amount of uric acid, (2) a high degree of acidity, (3) diminution of other salts, (4) diminution of urinary pigments.

TEST FOR URIC ACID—*Murexide Test.*—Evaporate a little urine to a small bulk, place 5 drops in a porcelain dish and add one drop of nitric acid ; then dry with heat. When almost dry add a small drop of ammonia. A positive reaction is obtained when a purple or rose colour develops at the edge of the drops.

There is no very convenient 'side room' method of estimating quantitatively the amount of uric acid passed per diem, but the following may be tried :—

QUANTITATIVE EXAMINATION FOR URIC ACID—(*Hopkin's Potassium Permanganate*

Method)—"To 100cc. of urine in a flask add 35 grammes of ammonium chloride. Allow to stand for 15 minutes. Insoluble ammonium urate is precipitated. Filter. Wash the precipitate with hot ammonium chloride solution. Open out the filter paper, and with hot distilled water wash the precipitate into a 100cc. flask. Dilute accurately to 100cc. with water. Pour into a beaker, add 20cc. strong sulphuric acid, and from a burette drop in $\frac{N}{20}$ permanganate solution made up as follows :—

Potassium permanganate. 7 9 grammes.
Distilled water, 1000cc.

1cc. corresponds to 0·00375 gramme of uric acid. The crimson fluid as it drops in is at first rapidly decolorised ; presently the colour disappears more slowly. When the liquid in the beaker retains a distinct pink colour for fifteen seconds, the end reaction is reached. Suppose 8cc. of permanganate solution have been added,

"In 100cc. of urine there were 8 × ·00375 grms. uric acid, *i.e.*, ·03 per cent." (*French*).

PURIN BODIES (Alloxur Bases)

These general names are given to uric acid and some bases of closely allied com-

position, *e.g.*, Xanthin ($C_5H_4N_4O_2$), Hypoxanthin ($C_5H_4N_4O$), Guanin ($C_5H_3N_4O\ NH_2$), and Adenin ($C_5H_3N_4\ NH_2$).

The quantity of uric acid is usually greater in amount, and much more constant, than that of the other bodies. These purin bodies are found in cases of disease where there is much destruction of nuclein, *e.g.*, Leucocythæmia.

Estimation of Purin Bodies.—A simple method of doing this is by the use of the Walker Hall Purinometer. Along with the instrument are provided directions for its use. The procedure is as follows :—

The purinometer consists of a tall, wide tube graduated in cc. which is closed below by a stop-cock separating it at will from a small reservoir of known capacity.

Two solutions are required :—

(1) Magnesia mixture	100 cc.
20 per cent. ammonia solution	100 cc.
Pure talc (in fine powder)	10 grms.
The magnesia mixture used consists of magnesium chloride crystals . .	110 grms.
Ammonium chloride . .	110 grms.
Ammonia	250 cc.
Water to	1000 cc.

(2)	Silver nitrate . . .	1 grm.
	Ammonia (strong) . .	100 cc.
	Talc	5 grms.
	Distilled water . . .	100 cc.

The stop-cock is closed and the instrument set upright in its stand.

Urine, freed from albumin, is poured into the glass cylinder up to 90cc. 20cc. of No. 1. magnesia solution is now added, and the instrument is vigorously shaken. The stop-cock is now opened and the precipitate of phosphates is allowed to settle in the reservoir. This takes about ten minutes. The tap is again closed, and No. 2. magnesia solution is added to the upper part of the tube until the total fluid in the tube reaches 100cc. A yellow precipitate of silver chloride and silver purin now forms. The tube is gently shaken backwards and forwards, and the instrument is allowed to stand for twenty-four hours when the bulk of the precipitate can be read off. A scale issued with the instrument enables this to be expressed in terms of the contained nitrogen.

In estimating the purin bodies, it is essential to investigate the purin content of the patient's diet so as to allow for the exogenous purins taken in with food.

CREATIN ($C_4H_9N_3O_2$)

is a normal constituent in small amount of urine, occurring more particularly in alkaline urine. By boiling with an acid it is converted into creatinin. It discharges the blue colour of Fehling's solution on prolonged boiling, but *unlike creatinin* it does not give a precipitate with zinc chloride, nor a colour reaction with nitro-prusside of soda and sodium hydrate.

CREATININ ($C_4H_7N_3O$)

is also a normal constituent of urine occurring specially in acid urine, being excreted to the extent of from 15 to 30 grains (1-2 grms.) per diem on an average diet. It is a dehydrated form of creatin, and when boiled with Fehling's solution for some minutes discharges the colour by reducing the cupric salt, but at the same time dissolving the cuprous oxide formed. When present in large quantity in the urine the above reduction may be brought about when testing for sugar.

In conditions accompanied by excessive muscular wasting, there is increased excretion of creatinin, and as a rule about one-half of the creatinin excreted is derived from the food.

TESTS.—*Weyl's Test.*—Make a fresh nitro-prusside solution by dissolving a few crystals of sodium nitro-prusside in an inch of warm water in a test tube. Mix this with an equal quantity of urine and add a few drops of caustic soda. The presence of creatinin causes the formation of a red colour which soon fades. If an excess of acetic acid be added and the mixture heated, a yellow colour, changing to green or blue, appears.

Jaffe's Test.—To an inch of urine in a test tube add half-an-inch of picric acid solution and render alkaline with caustic soda. Creatinin gives a brownish-red colour.

A quantitative method is based upon this last reaction, whereby a decinormal bichromate of potassium solution is used as a standard against dilution of the colour produced by Jaffe's test done with definite quantities.

CHLORIDES

Hydrochloric acid is combined in the urine in small amount with potassium, ammonium, and magnesium, but mainly with sodium. The amount of sodium chloride excreted daily is about 300 grains (15 grms.) and it ranks next to urea in amount as a dissolved solid constituent of the urine.

The output of chlorides is *increased* when large quantities of animal food are taken, also in rickets and cirrhosis of the liver.

Chlorides are retained in the tissues and *decreased* in the urine in chronic nephritis, before a uræmic attack, in fevers, especially pneumonia, until one or two days after the crisis, when the output is increased. Chlorides are also diminished in malignant disease, chronic gastric catarrh, and states of inanition.

DETECTION.—A test tube is half filled with urine. To this is added a few drops of pure nitric acid to hold the phosphates in solution. A 3 per cent. solution of silver nitrate is now added drop by drop (*e.g.*, 5-6 drops) till no more precipitate forms. In normal urine a thick, white, curdy precipitate should form at once. This test gives a rough estimation of the amount of chlorides present, because when the chlorides are much diminished only a faint hazy precipitate forms, and if no chlorides are present, no precipitate will form.

QUANTITATIVE ESTIMATION OF CHLORIDES.—This is conveniently done by Harvey's modification of the Volhard Method. The principle of this method consists in precipitating all the chlorides with an excess of

silver nitrate—silver chloride being formed. Ammonium sulphocyanate is now added to precipitate the silver nitrate which has not been used. By doing this in the presence of a ferric salt one can determine exactly the amount of cyanate solution required, because, as soon as all the silver nitrate has been precipitated as silver sulphocyanate, ferric sulphocyanate immediately forms, which is of a red colour and easily seen.

Reagents, etc., required :—

1. Siver nitrate (chem. pure) 29·042 grms. dissolved in 1 litre of distilled water. 1cc. of this solution = ·01 grm. of NaCl.
2. Solution of ammonium sulphocyanate in strength of 20cc. = 10cc. of the silver solution.
3. The indicator :—This is prepared by mixing 70cc. nitric acid (Sp. 1·2) and 30cc. of distilled water. Saturate this solution with crystaline ferric ammonium sulphate. Filter and store in a bottle with dropper stopper.
4. Burette.
5. Erlenmeyer Flask.
6. Distilled water.

METHOD OF PROCEDURE.—Fill a burette with the sulphocyanate solution.

Take 5cc. from a twenty-four hours' specimen of urine (albumin free), and place it in an Erlenmeyer flask. Add 20cc. distilled water. Add exactly 10cc. of the silver nitrate solution. The chlorides are precipitated as silver chloride.

2cc of the acid indicator are now added. Stir the mixture well, and add carefully the sulphocyanate solution till traces of red appear throughout the mixture. Stirring must be maintained while the cyanate is being added. By allowing the precipitate to settle, the red colour in the fluid can easily be seen. Calculate the number of ccs. of the cyanate solution used and divide the reading by 2, because 20cc. of this solution = 10cc. of silver solution. The figure now obtained is subtracted from 10, the number of ccs. of silver solution used. This figure gives the quantity of silver solution used to precipitate the chlorides in 5cc. urine and 1cc. of silver solution = ·01 grm. sodium chloride.

Therefore the number of ccs. used to precipitate the chloride multiplied by ·01 = amount of sodium chloride in 5cc. of urine. From this the amount per diem can easily be calculated.

N.B.—It is possible that the first drop of

the cyanate solution will give the red reaction. When this is the case, 10cc. more silver nitrate must be used because the first 10cc. have not been sufficient to precipitate all the chloride. Allowance has to be made for this in the calculation.

PHOSPHATES

Phosphoric acid occurs in the urine either combined with the alkalies, sodium, potassium, and ammonium (alkaline phosphates), or combined with calcium or magnesium (earthy phosphates). Three-fourths of the total phosphoric acid is combined with the alkalies, and these alkaline phosphates being very soluble are never precipitated. The remaining fourth occur as earthy phosphates, which are precipitated when the reaction of the urine becomes alkaline, especially when heated. This precipitate is distinguished from that due to albumin by the fact that it is completely soluble when a few drops of acetic acid are added.

Normally, 30-45 grains (2-3 grms.) of phosphoric anhydride are excreted daily, and physiological variations depend chiefly upon the amount taken in with the food. About $\frac{2}{3}$ of the ingested phosphorous is excreted in the urine, some also being excreted by the bowel.

Phosphates are *diminished* along with other solids in all renal diseases, in pregnancy, in gout, and in certain acute diseases, *e.g.*, pneumonia during the height of the fever.

Phosphates are *increased* in wasting diseases of the nervous system, neurasthenia, after hard manual work when the individual is not in good training, in cases where there is a rapid breaking down of nuclein—containing tissues, *e.g.*, in meningitis, in severe anæmia, in pneumonia, in severe diabetes, and in phosphatic diabetes.

It should be remembered that a great deposit of phosphates is rarely due to excessive excretion but simply, as a rule, to change in the reaction of the urine.

DETECTION.—Place half-an-inch of urine in a test tube and add a few drops of uranium nitrate, or uranium acetate solution with a few drops of sodium acetate solution. A greenish precipitate indicates the presence of phosphates.

QUANTITATIVE TEST OF PHOSPHATES IN TERMS OF PHOSPHORIC ACID.—Considerable ambiguity exists as to the clinical value of estimating the phosphates. The amount varies in most cases directly with that of uric acid.

The normal amount of phosphates eliminated in 24 hours is equivalent to about 15-75 grains P_2O_5 (1-5 grms.).

The amount of phosphates may be volumtrically determined thus :—

REAGENTS, ETC. :

1. Uranium nitrate solution—

   Take 35·495 grms. of uranium nitrate and dissolve in 1000cc. distilled water. This is standardised so that 1cc. = ·005 grm. P_2O_5.
2. 100 grms. acid sodium acetate are dissolved in 100cc. pure acetic acid and made up to 1 litre.
3. Tr. cochineal as indicator.
4. 10 per cent. potassium ferrocyanide solution.
5. Burette.
6. Beaker.

   Fill burette with uranium nitrate solution.

METHOD.—Take 50cc. of a twenty-four hours' sample of urine. Filter and place in a beaker, add 5cc. of the sodium acetate solution and 2-3 drops of cochineal.

Heat to boiling, and run in carefully the uranium nitrate solution. The end reaction is shown when the solution turns from the

brown colour to a green coloured precipitate. This can best be estimated by taking the mixture off the boil and allowing the precipitate to settle.

CALCULATION.—The number of cc. of uranium solution × ·005 = amount of P_2O_5 in 50cc. of urine. From this the daily amount can be calculated.

Instead of using cochineal as an indicator, potassium ferrocyanide solution may be taken, and drops of this arranged on a white slab. After each addition of uranium solution, a drop of the hot mixture is mixed with a drop on the slab. The end reaction takes place when the ferrocyanide solution turns brown. This reaction comes out rather later than that given with cochineal.

SULPHATES

Sulphuric acid is present in the urine daily to the extent of 30-45 grains (2-3 grms.); of this about nine-tenths are combined with potassium and sodium, while one-tenth is combined with aromatic substances like phenol and indol (ethereal sulphates). The total sulphates are increased when sulphur is administered, in conditions which cause breaking-down of proteins, *e.g.*, arsenic and phosphorus poisoning, and prolonged febrile

diseases like typhoid, pneumonia, and rheumatic fever; and in conditions like diabetes where an excess of animal food is eaten.

The ethereal sulphates are increased relatively to the others in conditions which cause increased intestinal putrefaction and the formation of phenol, indol, etc. Similarly, they are diminished by calomel and other intestinal antiseptics, and by a milk diet. They are increased greatly, and may completely replace the other sulphates after poisoning by carbolic acid.

TEST.—Place two inches of urine in a test tube and render it strongly acid with acetic acid to prevent precipitation of the phosphates; add a small quantity of a solution of barium chloride and a white precipitate comes down due to the preformed sulphates; filter off this precipitate; add to the filtrate a few drops of strong hydrochloric acid and heat it; a second precipitate forms due to the setting free of sulphuric acid from the ethereal sulphates.

QUANTITATIVE ESTIMATION OF SULPHATES.—From the above test a rough idea of the comparative amount of the preformed and ethereal sulphates may be obtained. There is no simple titration

method of estimating them, and the following method is troublesome to carry out.

(a) Total Sulphates :—

50cc. of urine, 50cc. of distilled water, and 10cc. of dilute hydrochloric acid are mixed, raised to the boiling point, and a 5% solution of barium chloride added till the white precipitate formed grows denser (about 10cc.); the mixture is kept warm for two hours, allowed to cool and stand for twenty-four hours, and then filtered through an ash-free filter paper. The precipitate on the water is thoroughly washed with distilled water, then with alcohol, and incinerated in a platinum capsule. After cooling it is weighed, and for every 100 grams. of barium sulphate weighed, 41·99 grams. of sulphuric acid were originally present.

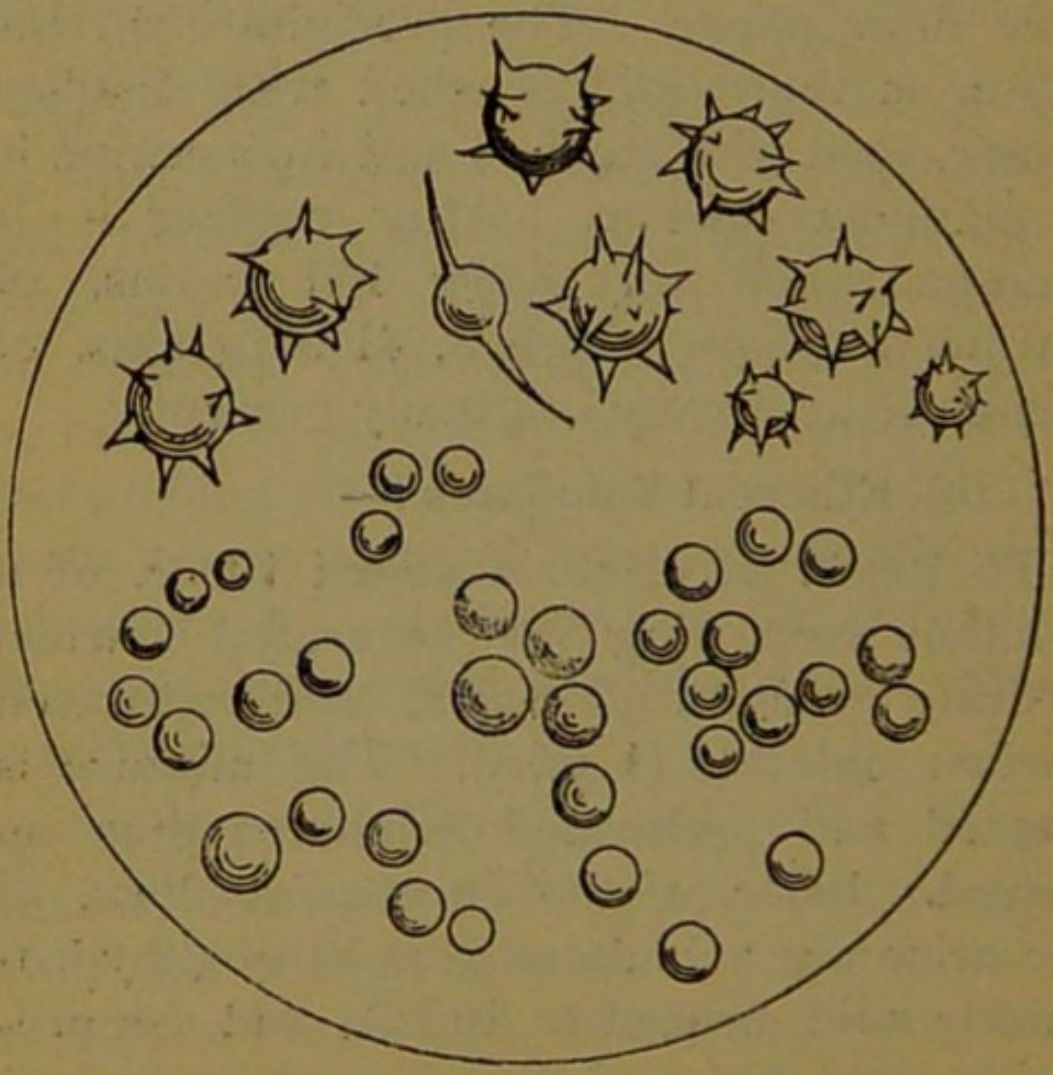
(b) Ethereal Sulphates :—

To 100cc. of urine is added 100cc. of a solution consisting of saturated barium hydrate solution (2 parts) and saturated barium chloride (1 part). The mixture is filtered and preformed sulphates thus removed. 100cc. of the filtrate, *i.e.*, 50cc. of the urine are acidulated with strong hydrochloric acid, heated to 100°C., and the precipitate which then falls (ethereal sulphates)

is treated as above described in the estimation of the total sulphates.

URATES

IN HEALTH amorphous urates, consisting of sodium, potassium, ammonium, and calcium, in varying proportions, combined with uric acid, may occur after profuse sweating and violent exercise in cold weather, and after prolonged abstinence from food or after an injudicious diet. Crystalline urates of soda



URATES OF SODA URATES OF AMMONIA

or ammonia—"hedgehog" or "thorn-apple" crystals—may occur in health, but if they become permanent grave disorders may follow. As a rule, amorphous urates are of little clinical significance, as they are unlikely to be deposited and cause irritation in the kidneys, ureter, or bladder as long as the urine is of the temperature of the body. The crystalline, however, are not so harmless.

IN DISEASE amorphous urates indicate some amount of fever; they are frequently seen in cases of dyspepsia, catarrh, and various organic diseases of heart, lungs, or liver.

The deposit of urate of soda, taking place, as it does within the urinary passages, may lead to the formation of a calculus; urate of ammonia may be found mixed with phosphates in phosphatic calculi.

OXALATES

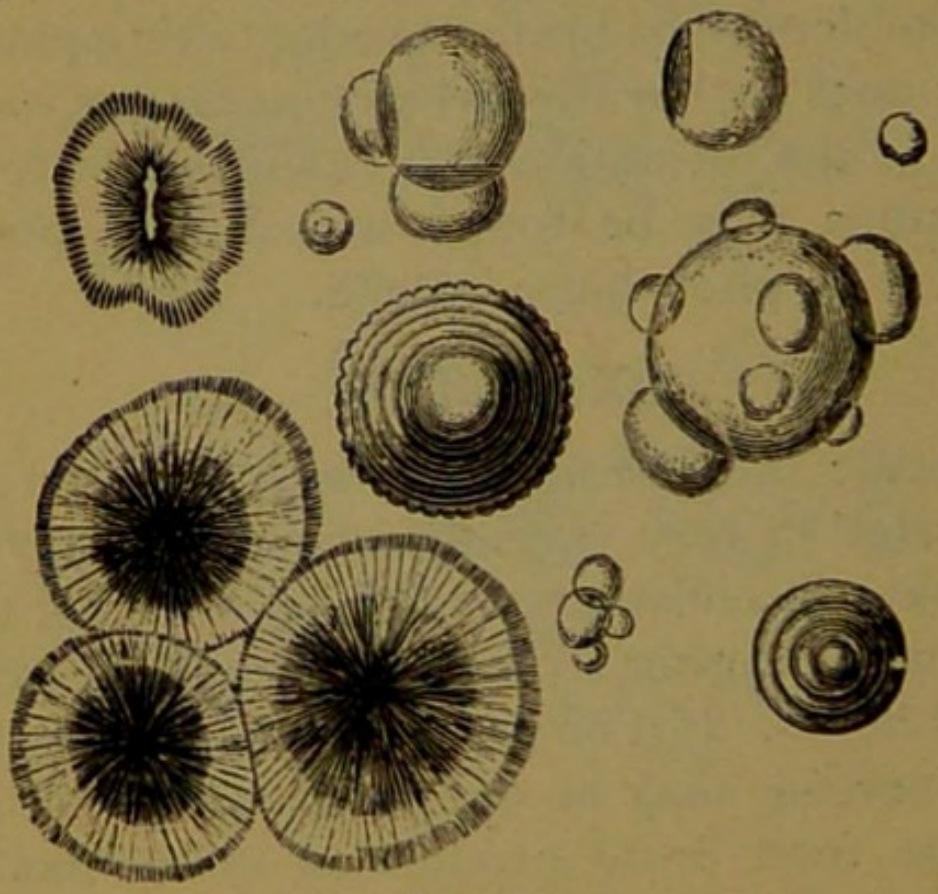
Oxalic acid combined with calcium is excreted in very varying amount, but forms probably a constant constituent of urine, the quantity ranging from a trace up to ·1 grm. in 24 hours. If it be present in large amount, the increase may be due either to the nature of the food or to production in the body. Food rich in nucleins, *e.g.*, sweetbread,

gelatin, and probably proteins, increase it. A condition known as idiopathic oxaluria is characterised by excretion of excessive amounts of oxalate with pain in the lumbar region, hypochondriasis, and loss of weight. Oxalates in the urine are increased by the use of certain vegetables and fruits, *e.g.*, rhubarb, carrots, apples, gooseberries, strawberries, etc.

There may be a tendency to calculus formation when oxalates are found in the urine.

LEUCIN ($C_6H_{13}NO_2$)

may be derived from auto-digestion of



Leucin

organs, *e.g.*, the liver, or by defective cleavage of the amino-acids produced by normal digestion in the small intestine. It is especially found in the urine in severe diseases of the liver, *e.g.*, acute yellow atrophy, phosphorous poisoning, advanced cirrhosis, and also in some general diseases like pernicious anæmia and leucocythæmia. It is found in the form of small, fatty-looking, balls with radial and concentric markings; these are not unlike balls of ammonium urate, carbonate of lime, or oxalate of lime in appearance, but, whilst leucin is soluble in a mixture of warm alcohol and ammonia, the others are not.

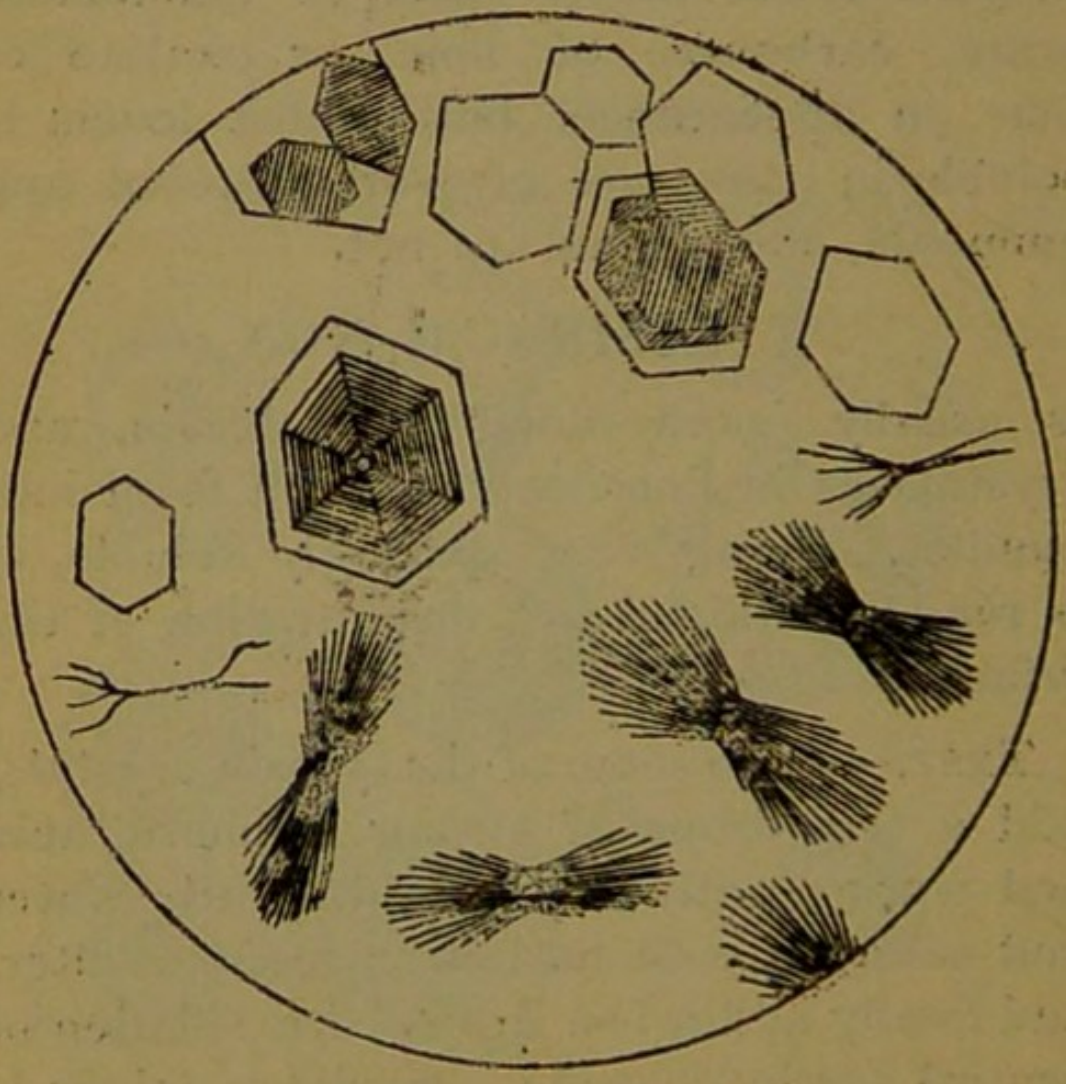
TYROSIN ($C_9H_{11}NO_3$)

is usually found along with leucin, and crystallises in fine needles which form into bundles. Being very sparingly soluble, it is readily obtained by concentration of the urine.

TEST.—Take some of the tyrosin crystals, add a few drops of strong sulphuric acid and warm gently; then add a little water and saturate with barium carbonate, filter, and finally add a few drops of a solution of neutral ferric chloride. A violet colour is produced.

CYSTIN ($C_3H_6NSO_2$).

occurs in thin, transparent, hexagonal plates with very characteristic appearance, and is a cleavage product of proteid digestion, which some persons cannot oxidise and, therefore, excrete in the urine. This peculiarity is often hereditary. Cystin may be precipitated from a urine that contains it by free acidulation with acetic acid, it then forms a greyish deposit.



TYROSIN CYSTIN

PHENOL

Phenol or carbolic acid (C_6H_5OH) and cresol ($C_6H_4OH.CH_3$) are two closely allied aromatic bodies formed by protein decomposition, and found in human urine in the form of phenol potassium sulphate ($C_6H_5OSO_6K$). They are formed mainly in the intestinal canal, and are joined with sulphuric, and to a less extent glycuronic acids, combined with a base, usually potassium, and excreted almost entirely by way of the urine. About 2-3 mgms. are excreted daily. The amount is increased by all conditions that intensify intestinal putrefaction, *e.g.*, chronic obstruction, peritonitis, ulceration of the bowel, and even constipation; also by septic processes elsewhere in the body, *e.g.*, large abscesses and empyema.

TEST.—To an inch of urine in a test tube add bromine water drop by drop; if phenol be present, a yellowish-white precipitate appears, temporarily at first, but becoming permanent when the bromine water is added in excess.

Pyrocatechin and **Hydroquinone** are both derivatives from phenol, the latter only appearing when phenol has been administered by the mouth, or absorbed through

the skin to excess. They are crystalline bodies, readily soluble in the urine, and oxidise on exposure to the air giving the urine a dark-drown or black colour, and producing so-called carboluria. Like phenol, they are excreted in combination with sulphates.

TEST.—Pyrocatechin is precipitated by acetate of lead; it reduces Fehling's solution on prolonged boiling. If a solution of tartaric acid and ferric chloride be alkalinised with ammonia and added to urine containing pyrocatechin, a cherry-red colour is produced which becomes green on free acidulation with acetic acid.

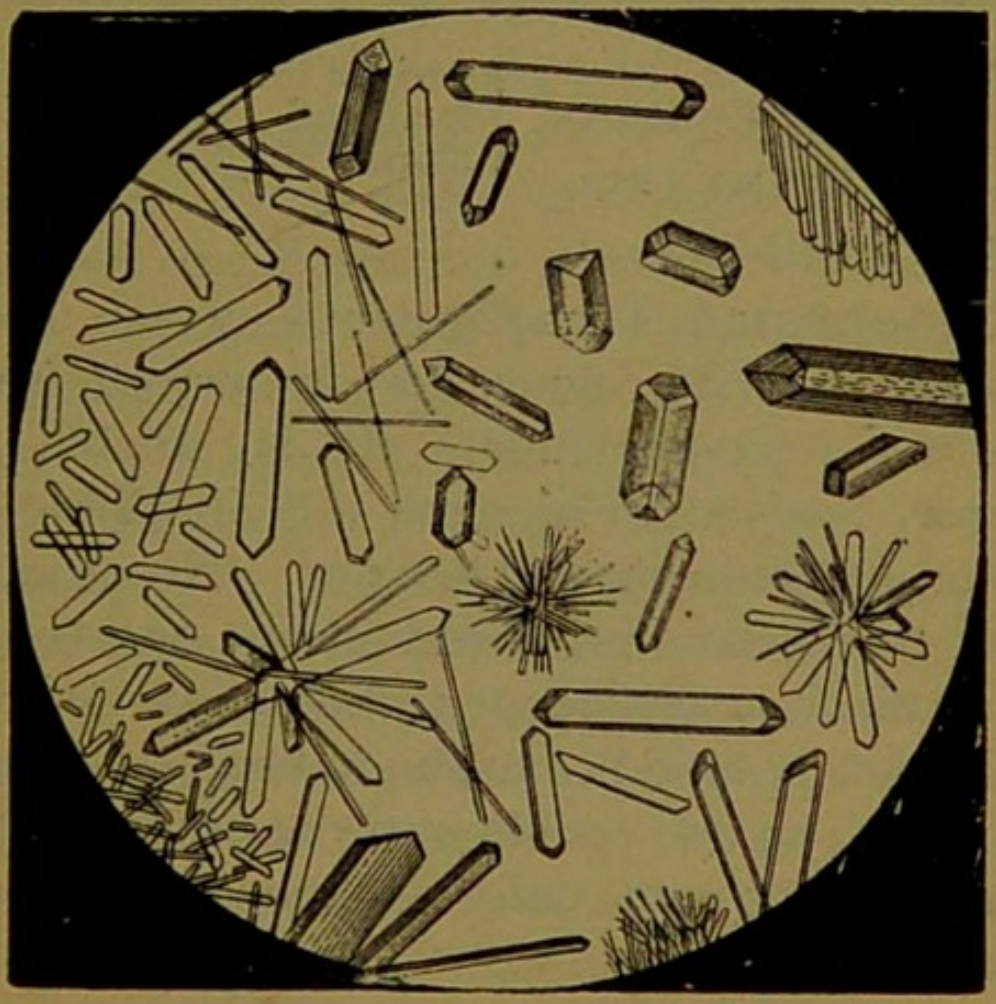
Hydroquinone is not precipitated by acetate of lead, it reduces Fehling's solution on prolonged boiling. When it is acted on by ferric chloride, quinone is produced which may be recognised by it peculiar odour.

Hippuric Acid occurs in the urine of a healthy man on an average diet to the extent of about ·7 grms. per diem. It is partly derived from the aromatic bodies present in vegetables, and the output is increased by the taking of benzoic acid.

The urinary output of hippuric acid is increased in diabetes, the febrile state, and

certain cases of abnormal liver function.

PREPARATION.—Treat very fresh urine with HCl in excess, concentrate and set it aside to crystalise. Dissolve the crystals in hot water and again crystalise by cooling. The crystals may be distinguished from those of uric acid by being soluble in alcohol and hot water, the absence of the murexide test, and by the microscopic appearance of the crystals.



HIPPURIC ACID

URINARY PIGMENTS

Some pigments occur naturally in the urine, some exist as chromogens, *i.e.*, colourless substances which, on addition of chemical re-agents or as the result of exposure to light and air, develop colouring properties.

The following table shows the chief pigments that may be excreted in the urine :—

As Pigments		*As Chromogens*
UROCHROME.		INDOXYL.
UROËRYTHRIN.		SKATOXYL.
UROBILIN.	derived from the blood.	ALKAPTON.
HÆMATOPORPHYRIN.		MELANIN.
BLOOD PIGMENTS.		
BILE PIGMENTS.		

Urochrome is the pigment to which urine mainly owes its colour, and is derived from protein decomposition. It is soluble in water, less so in alcohol, and insoluble in ether, chloroform, and benzine. No absorption bands are produced in the spectrum. With nitric acid it deepens the yellow colour. It is closely allied to urobilin, because either of these is convertible into the other. The amount excreted daily is about 8 grains (·5 grms.)

Uroërythrin may be present in some normal urines. It causes the salmon colour

of urates seen when they are cooled and deposited. This pigment is increased by a rich meat diet, profuse sweating, circulatory disturbances of the liver, and some digestive irregularities.

Urobilin, or its chromogen urobilinogen, occurs in normal urine. When the pigment is present in large quantity, it gives a deep yellowish-brown colour to the urine with a bright yellow tint in the froth.

Morris advises the use of Schlesinger's Test. Place 10cc. of acid urine in a test tube and add 5-6 drops of Lugol's iodine solution to convert any urobilinogen into urobilin. Now make up a saturated solution of zinc acetate in absolute alcohol, and add an equal quantity of this solution to the urine in the test tube. The test tube is now held against a dark background and examined with transmitted light. If the reaction be positive, a green fluorescence appears in the mixture, the intensity depending on the quantity of urobilin present.

Care must be taken to see that the glassware has no fluorescing compound in it, nor a trace of eosin in or on the tube.

It has a single characteristic absorption band in the spectrum between the green and the blue.

Hæmatoporphyrin, an iron-free derivative of blood, sometimes occurs in the urine after large doses of sulphonal, trional, and other drugs possessed of a hæmolytic action. The urine is coloured a burgundy-red when hæmatoporphyrin is present. This substance is sometimes also present in the urine of patients suffering from carcinoma, cirrhosis of the liver, febrile and septic diseases, chronic syphilis, and lead poisoning.

TEST.—The phosphates in the urine are precipitated by 10 per cent. sodium hydrate. This precipitate carries down the pigment. The precipitate is filtered off and the pigment is now dissolved in acid alcohol (HCl 5cc. +95cc. alcohol). By means of the spectroscope two absorption bands are seen, one just to the left of D., and the other, broader band, between D and E.

Blood and Bile pigments are described later. See pages 84 and 87.

Indoxyl Compounds.—Indoxyl is produced by oxidisation of indol, and when excreted in combination with sulphuric acid is known as indican. Most of the urinary indican is in the form of potassium indoxyl-sulphate, some is also present as potassium

indoxyl-glycuronate. Indol is derived, like phenol and skatol, from intestinal putrefactions of proteins. It is greatly increased in all conditions which augment intestinal decomposition. The indoxyl compounds do not, however, colour the urine so long as they are combined with a base. Occasionally the indican breaks up and then becomes oxidised to indigo-blue or indigo-red, which imparts to the urine a green or red colour and deposits in a precipitate.

TEST.—The test depends on freeing the indican from its base and oxidising it to indigo. Place two inches of urine in a test tube, add an equal bulk of strong hydrochloric acid and one inch of chloroform. Then add a crystal of chlorate of potash, or a few drops of a solution of bleaching powder, or H_2O_2 to act as an oxidising agent. Close the tube and invert it several times. The chloroform is tinted blue or reddish by the indigo.

N.B.—If too much oxidising agent be used, the pigment is again decolorised.

Skatoxyl Compounds.—Skatol is derived from the same source, and excreted in the fæces and urine in the same combinations as indol.

Test.—When a strong acid is added in the amount of 10 per cent. to a urine containing skatol the latter is broken up and oxidised by the air to form a red colour. This often appears when the urine is tested with nitric acid. The oxidation may be assisted by adding a minute crystal of potassium chlorate. The red colour in this case is not taken up by chloroform, which distinguishes it from indigo-red.

Melanin.—In the urine of people with pigmented new growths, and those with wasting diseases like phthisis, a pigment sometimes occurs which darkens slowly on exposure to the air. Bromine water added to urine containing melanin produces a yellow precipitate which slowly blackens.

Alkaptonuria is a term applied to a rare condition due to a disturbance in metabolism. The condition may occur in apparently healthy people, but seems to be increased, when established, by a meat diet and after the administration of tyrosin.

The urine shows certain characteristics :—

1. When the urine is voided it is very acid, and of a normal colour.
2. On standing, oxidation quickly takes place, rendering the urine a reddish-brown colour, changing later to black.

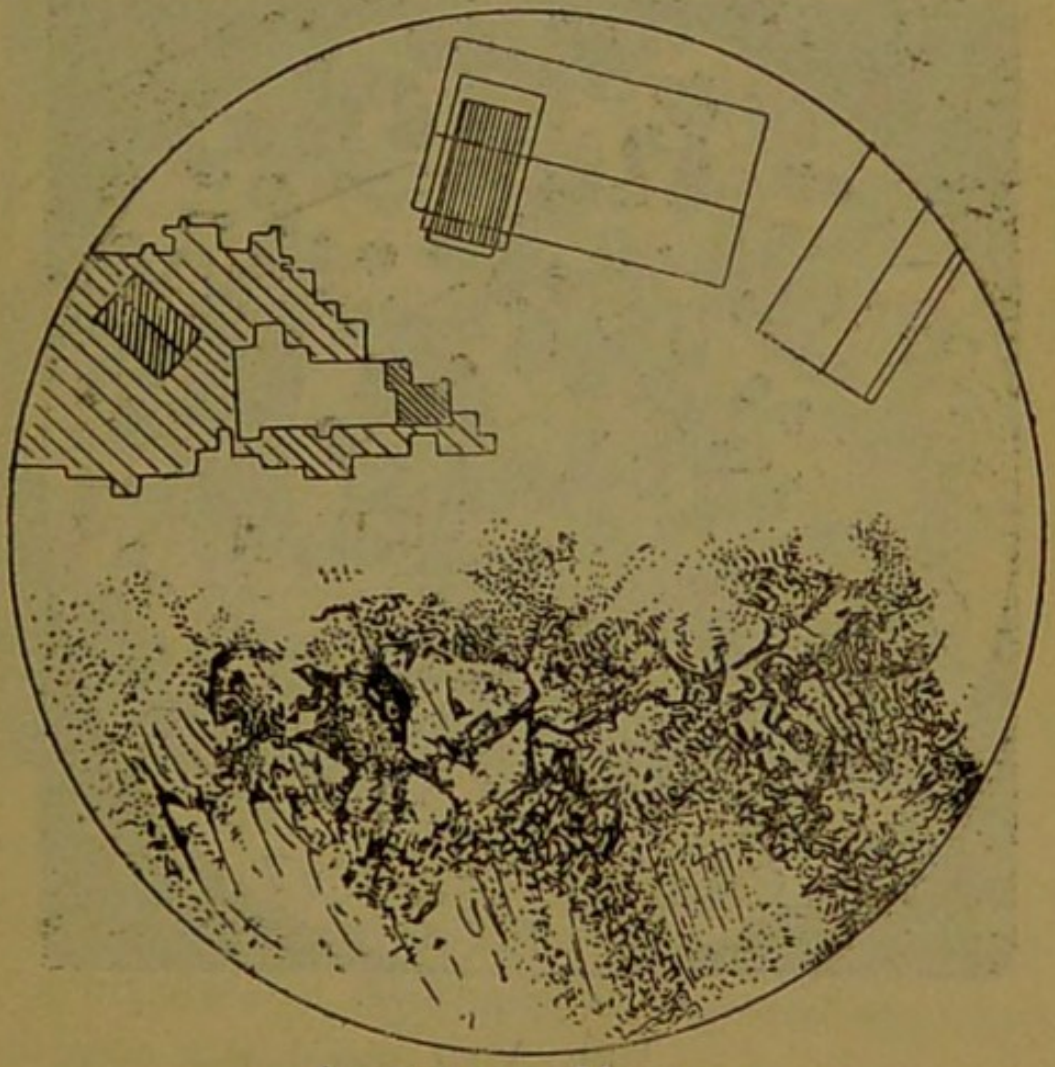
When the urine is rendered alkaline, the colour changes take place more rapidly.

3. The urine reduces copper, but not bismuth. It does not ferment yeast, nor deviate polarised light.

These colours and reduction reactions are due to alhapton bodies, one of which is homogentisic acid.

URINARY DEPOSITS

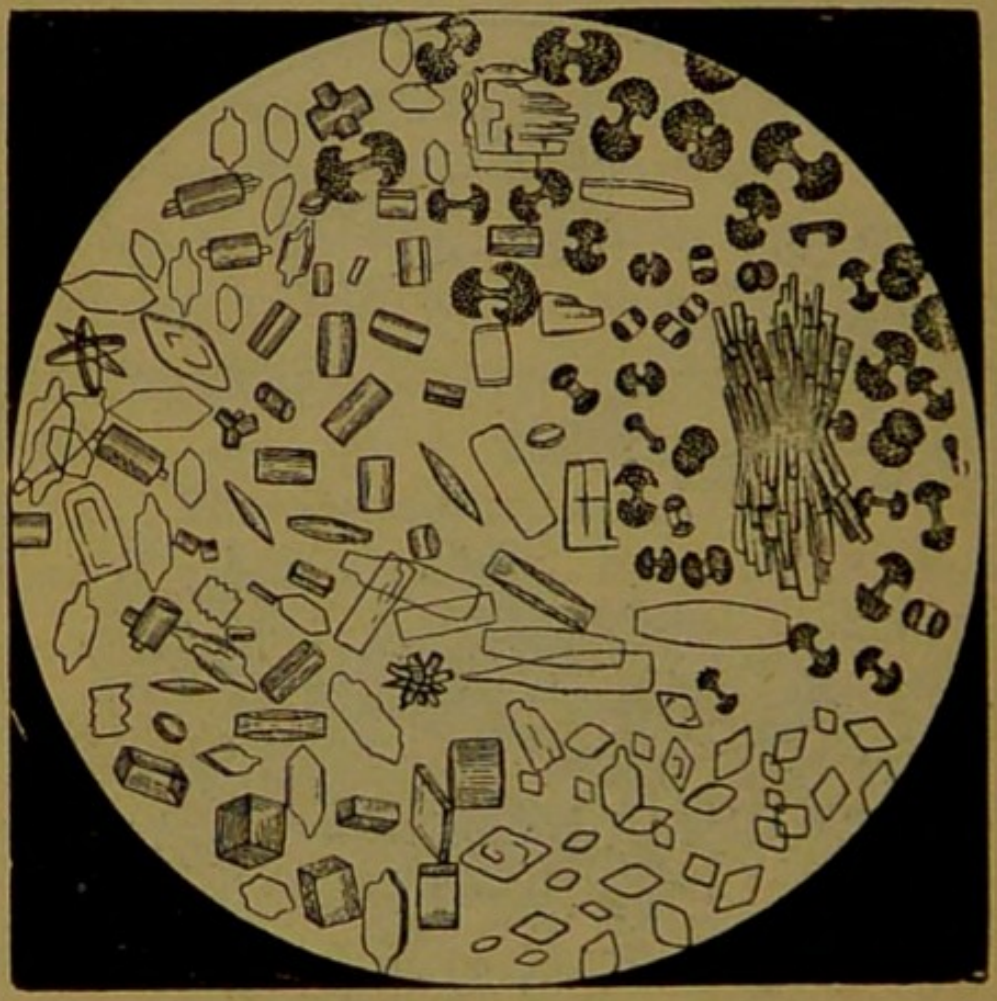
Amorphous Urates.—A heavy deposit of urates is common in all febrile conditions,



AMORPHOUS URATES

and may occur in normal individuals after hard exercise. Urates carry down a large quantity of urinary pigment. They have a pinkish colour, and when heated redissolve at once. They occur in acid urines. Under the microscope, urates have an amorphous appearance of brownish-yellow balls with spines. They consist largely of acid sodium urate.

Uric Acid.—Uric acid occurs in acid urine, and is commonly precipitated in



Uric Acid

urines of low specific gravity where the excretion of urinary salts is deficient. When it occurs in urines of normal specific gravity, it usually signifies a strong gouty tendency. Uric acid crystals may be recognised in a specimen glass as small red grains like cayenne pepper at the bottom and sides of the glass.

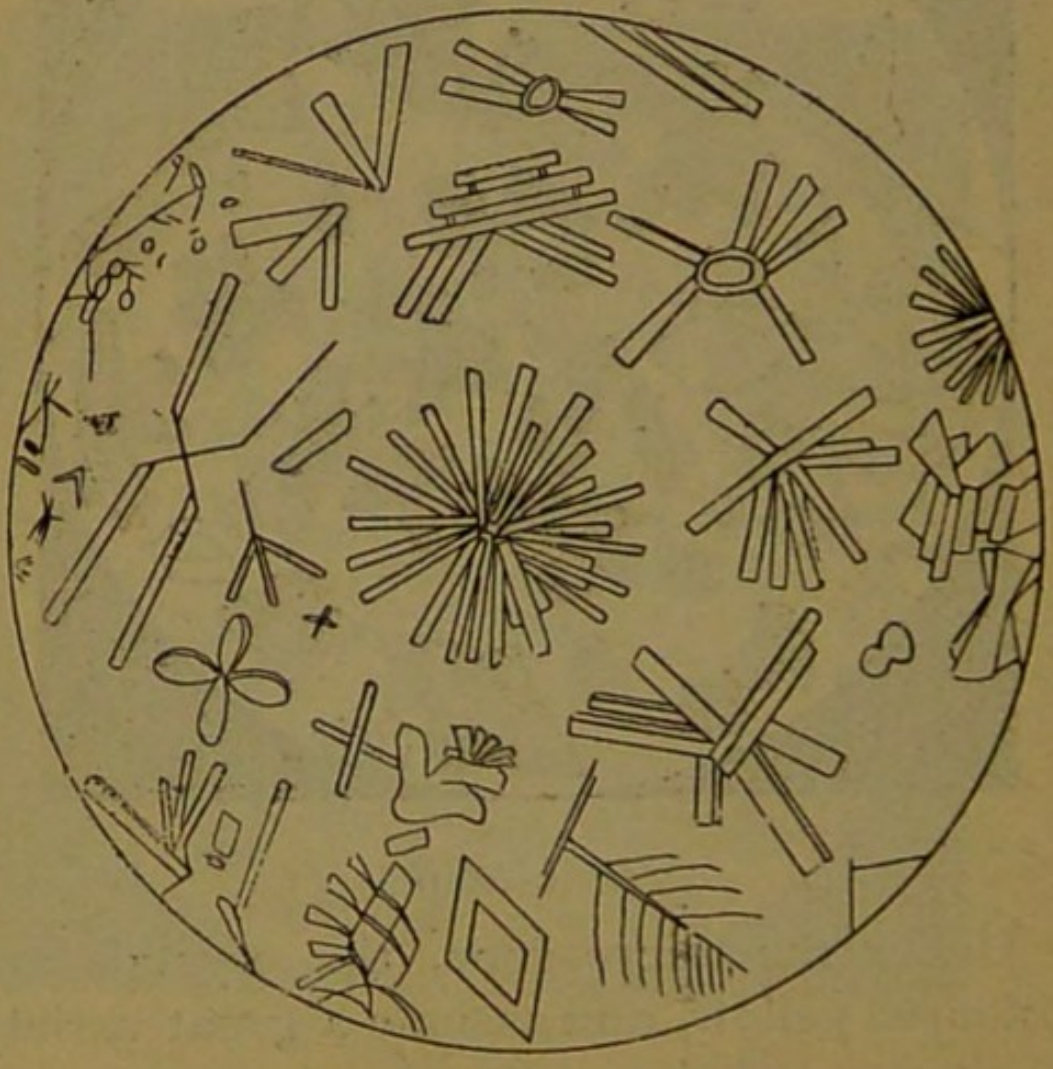
URIC ACID

Under the microscope, the crystals are coloured yellow, and assume a great variety of shapes and sizes.

The type of crystal most frequently seen is known as the whetstone or barrel-shaped, others have a more pointed extremity. Needles and rod-shaped forms may also be seen. Crystals may arrange themselves together in a fan-shaped way.

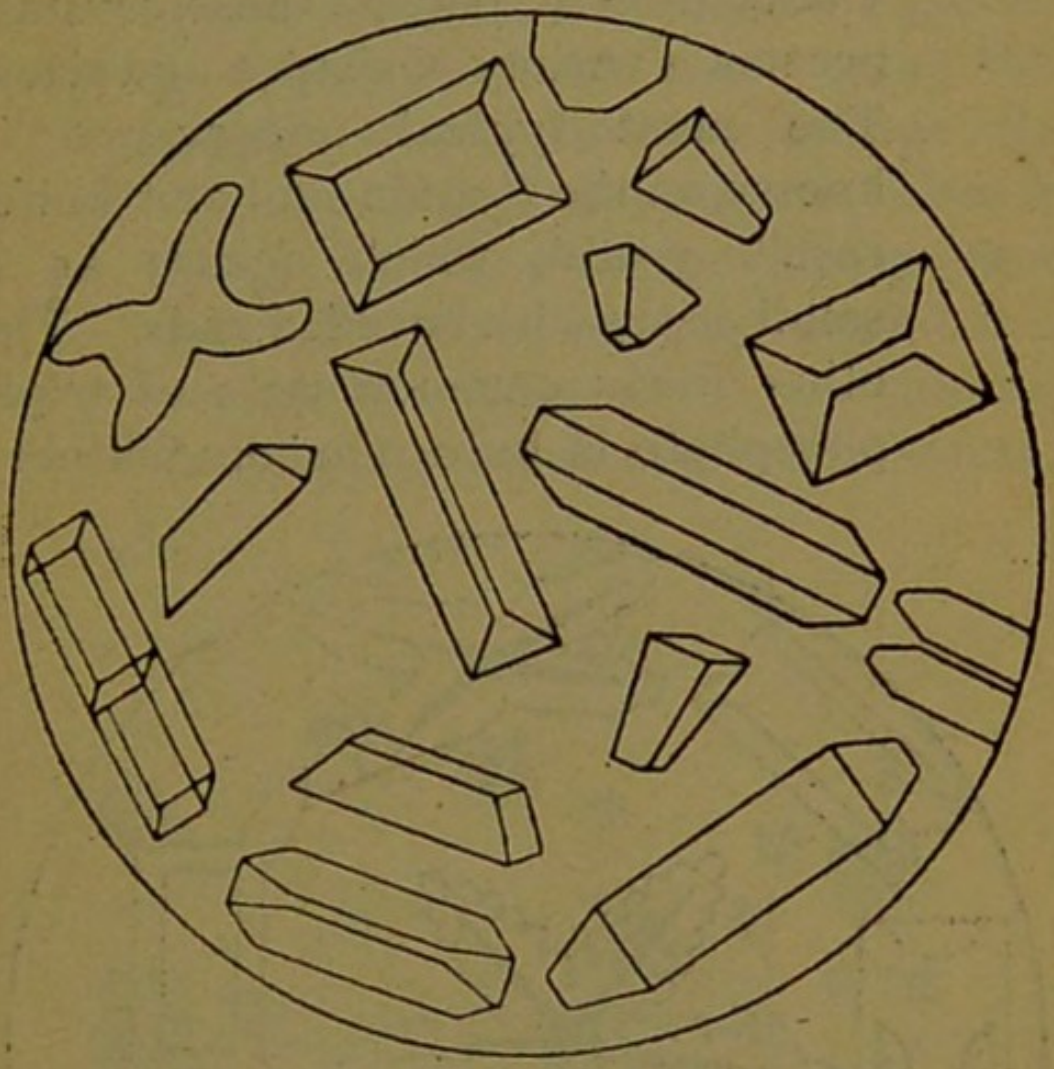
Phosphates are deposited in the urine in various forms.

1. AMORPHOUS PHOSPHATES are found in alkaline urine as a whitish almost pus-like deposit. Under the microscope



PHOSPHATES OF LIME

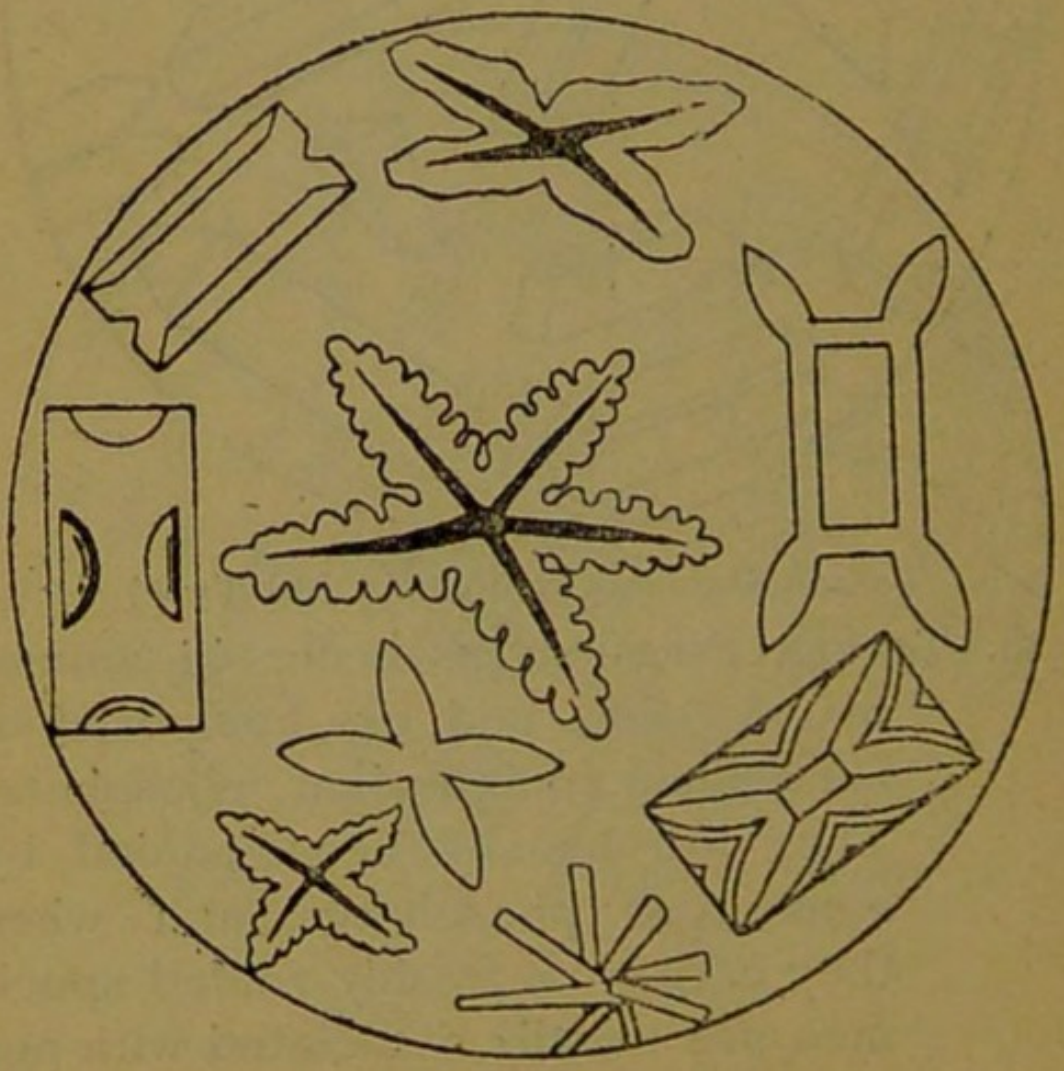
they appear as amorphous granules, which do not dissolve on heating, and occur in an alkaline urine.



TRIPLE PHOSPHATES (Coffin Lid Type)

2. TRIPLE PHOSPHATES occur in ammoniacal urine, and are composed of ammonium magnesium phosphate. They are usually accompanied by amphorous phosphates, and when they occur in a freshly-voided specimen are usually associated with pus and micro-organisms, all accompani-

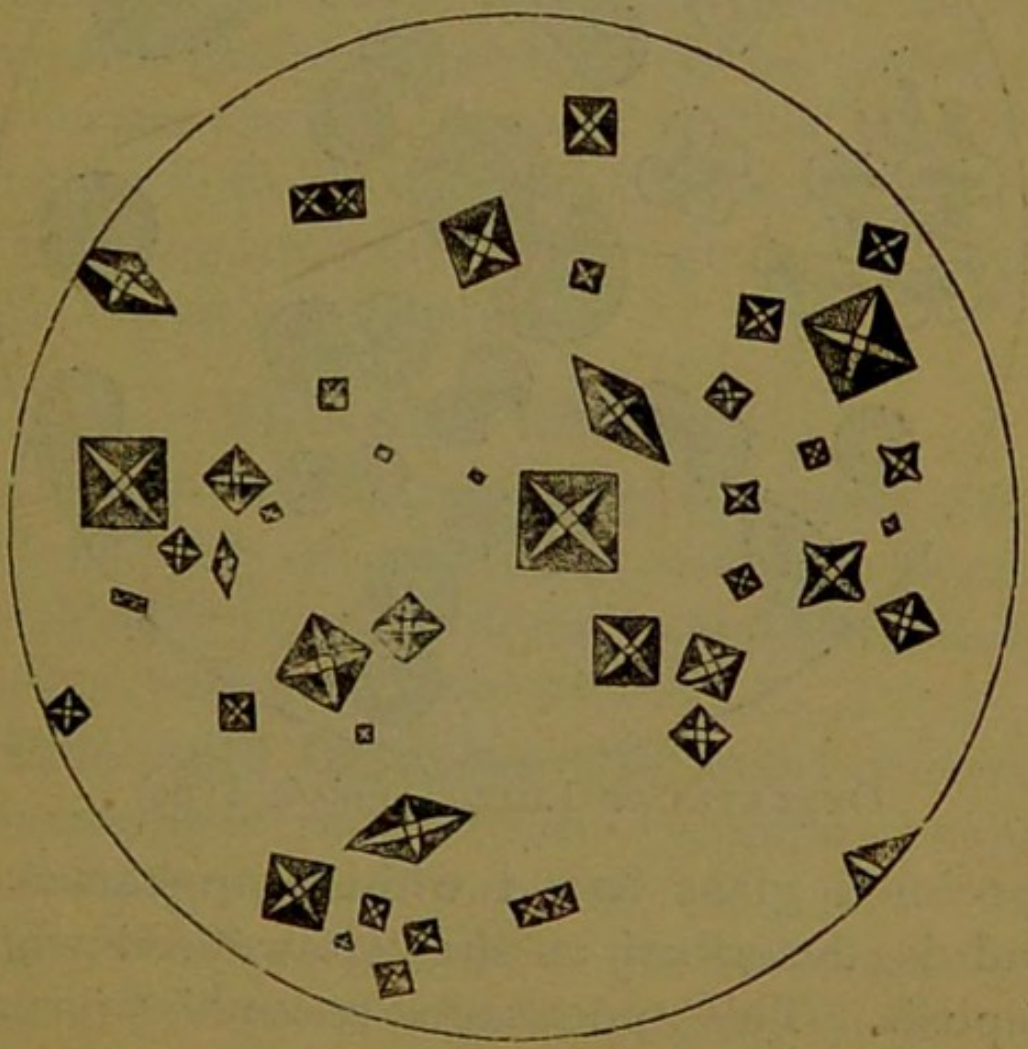
ments of an ammoniacal urine. The presence of triple phosphates in stale urine has no clinical significance. Under the microscope these crystals present a variety of shapes and sizes. The most typical appearance is known as the "coffin-lid" or "knife-rest" crystal, which occurs as a small prism with bevelled ends. The other fairly common type of triple phosphate is known as the feathery



TRIPLE PHOSPHATES (Stellar Phosphates)

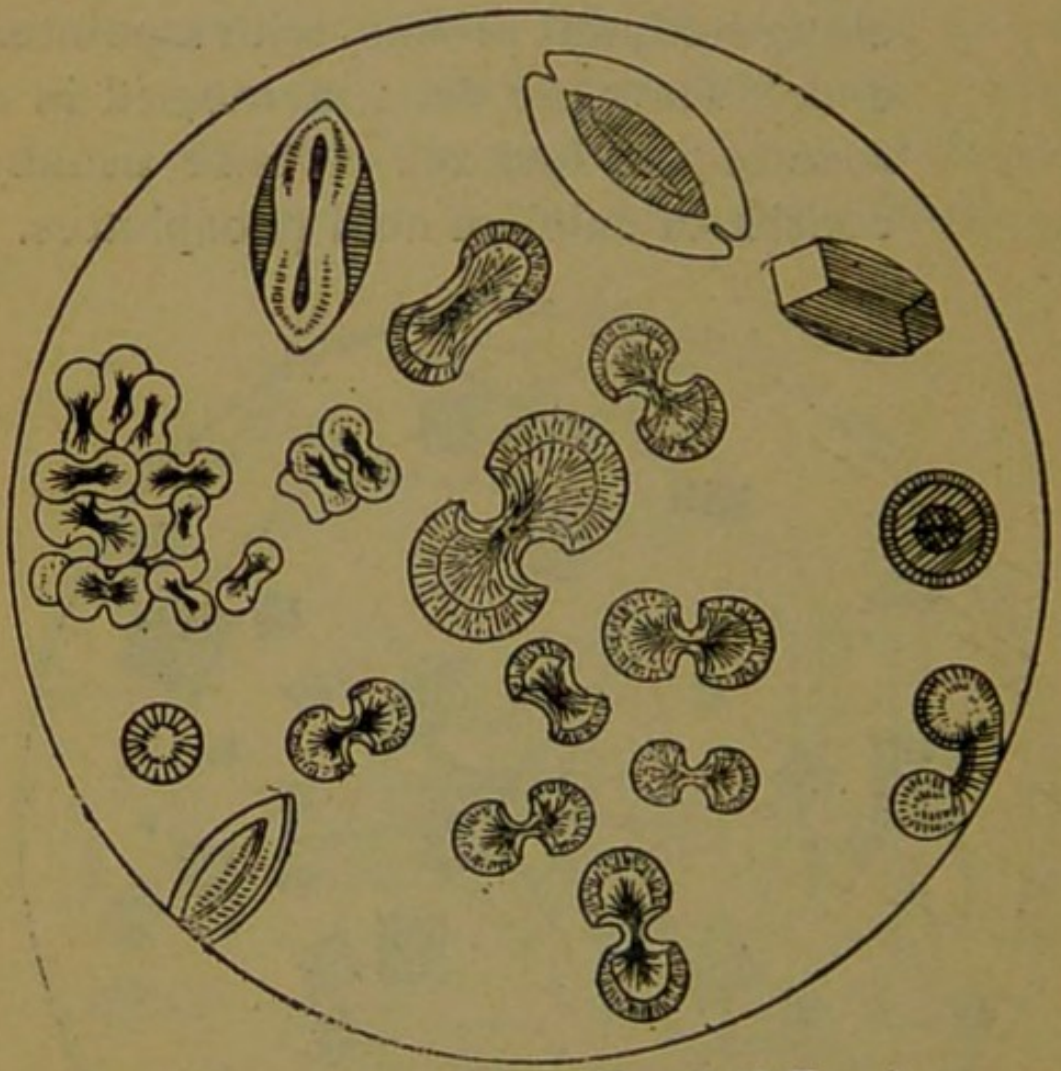
phosphate, resembling the branching of a fern.

3. STELLAR PHOSPHATES may be found in healthy urines, but more frequently in connection with some blood disease. Under the microscope the crystals usually assume the form of elongated, flat prisms, with a pointed end. They are often arranged in a rosette-like fashion. These usually consist of calcium acid phosphates.



CALCIUM OXALATE (Envelope Type)

Calcium Oxalate crystals occur more frequently in acid than in alkaline urine. They may be deposited in a normal urine after standing for some time. More commonly they are found after the ingestion of certain fruits and vegetables. A profuse deposit of oxalates at the bottom of a



OXALATES OF LIME (Dumbell Type)

specimen glass has a whitish appearance, and is described as the "powdered wig" deposit. This appearance resembles pus at times.

Under the microscope the typical crystals show an "envelope"-like appearance. Dumb-bell shaped forms are also met with. The oxalate crystals are very small and require a high power to identify them with certainty.

Cystin is a very rare urinary deposit. Under the microscope the crystals are flat, colourless, six-sided discs.

Many foreign bodies may be found in urinary deposits. It is important to remember when examining slides that marks on cover glasses or slides may be mistaken for some curious deposit. This possible mistake is easily eliminated by—

1. Using absolutely clean glasses.
2. Focussing carefully.

URINARY CALCULI

Urinary calculi are seldom composed only of one type of substance, different layers being added under different conditions as to acidity, sediment present, etc.

Uric Acid Calculus.—By many authors this is considered to be the commonest type of urinary stone.

It varies in size from a pea up to a large egg. It is usually fawn or greyish-yellow in colour. The surface is smooth or finely

granular. It is hard, breaks easily, and the cross section shows a laminated appearance, the layers differing in colour.

TESTS.—Add a few drops of HNO_3 to some of the powdered stone, and evaporate —a pink stain results, cool, and add a few drops of NH_3 to the stain which is changed to purple.

Uric acid is soluble in alkalies.

Oxalate of Lime.—Oxalate stones may be small and smooth, or large and rough. They are brownish in colour due to the bleeding they set up, and in many cases the surface is nodular, hence the name "mulberry" stone. They are very hard and heavy.

TEST. — The powder from the stone is soluble in strong HCl, but not in acetic acid, which differentiates it from phosphates.

Phosphatic Stones.—Calculi may be formed of calcium phosphate. These vary in size, are hard, white and smooth. More commonly a phosphatic calculus consists of a deposit of triple phosphates from alkaline urine around a core of oxalate of lime, or uric acid. In this latter case, the calculus is white with an uneven surface and very friable.

TEST. — Both calcium phosphates and

triple phosphates are soluble in acetic acid. To the solution so formed add a little ammonium molybdate solution and a yellow precipitate forms which is again soluble in ammonia.

TUBE CASTS

A tube cast is the name usually given to small cylindrical bodies shed from the diseased tubules of the kidney, and passed in the urine. Casts are to be found in the deposit of a urine from diseased kidneys, and occur in various shapes and forms depending on the pathological state of the kidney at the time.

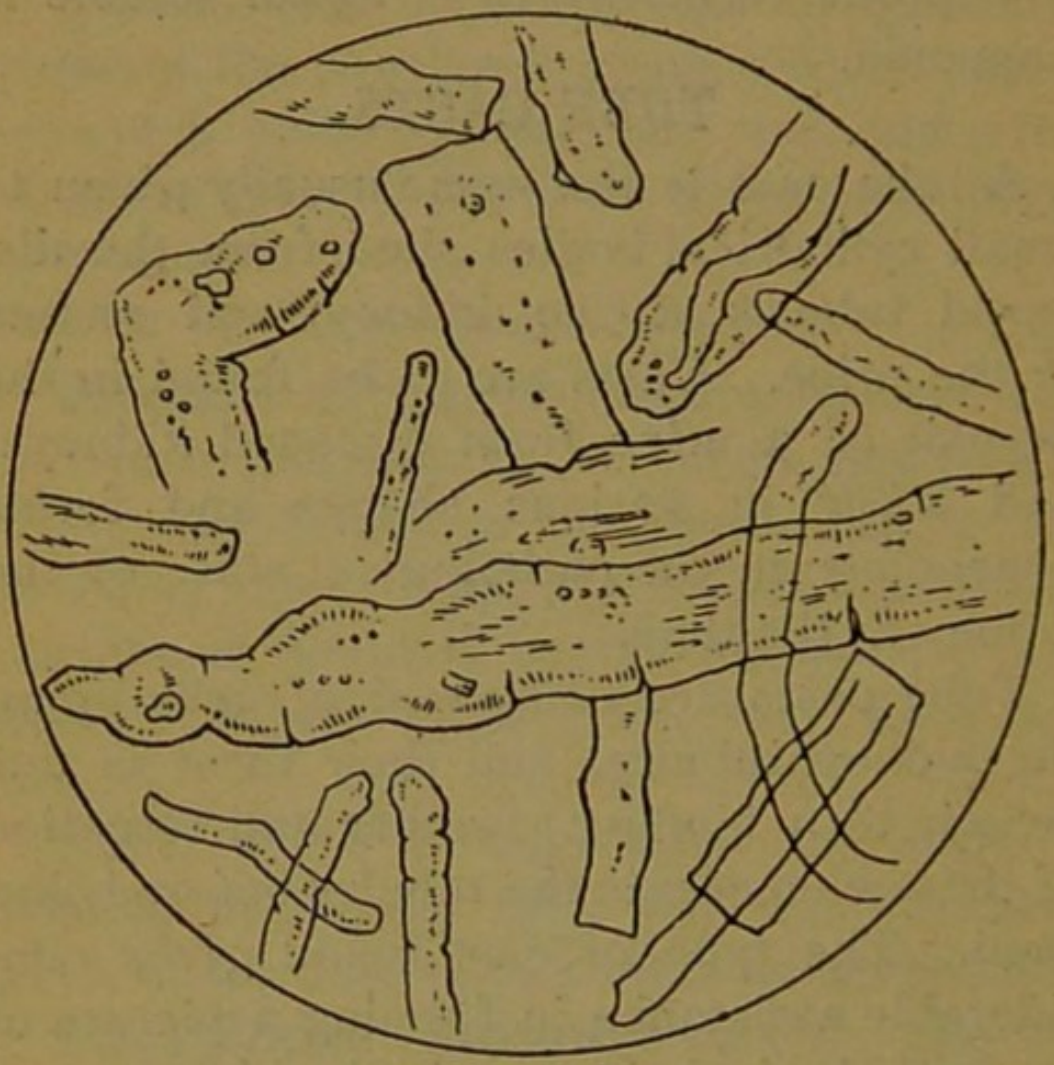
Tube casts are composed of cells lining the kidney tubules, and their form is due largely to a binding together and retention of these elements in the tubules before being shed. The type of cast found gives considerable assistance in forming a picture of the pathological changes in the kidney.

HYALINE CASTS.—This type of cast is the most frequently found.

(1) Formed by a concentration of albumin in the tubules.

(2) Appearance. Under the microscope is seen a small, highly refractile cylindrical tube, quite clear with clean-cut edges.

(3) Significance. These may occur in any urine in which albumin is, or has been present.



HYALINE CASTS

EPITHELIAL CASTS.—

(1) Formed by a shedding of the tubular epithelium which sticks to the hyaline casts, and is voided before degenerative changes take place.

(2) Appearance. An aggregation of epithelial cells is seen in tube-like form.

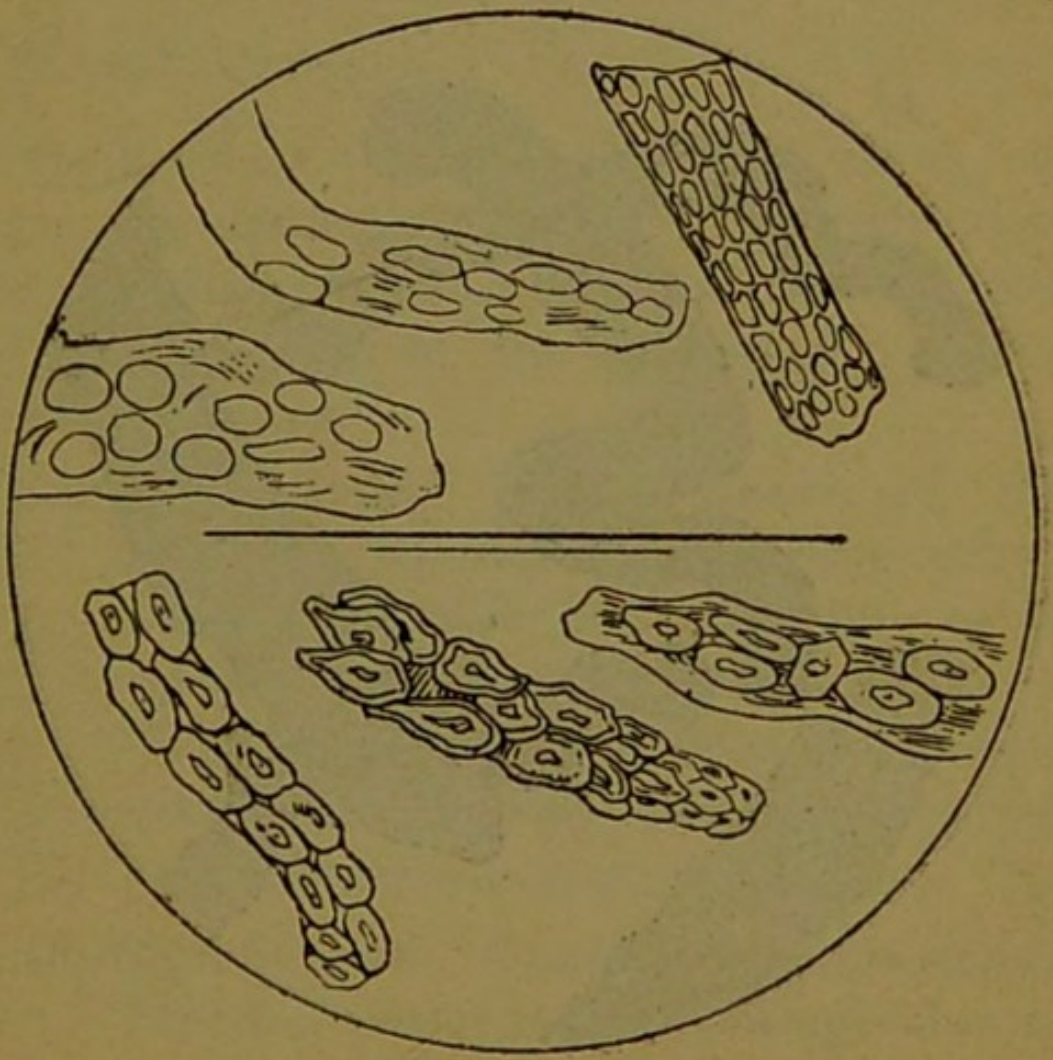
Each cell-outline can be distinguished as well as the cell-nucleus.

(3) Significance. They are present in the early stages of acute nephritis.

BLOOD CASTS.—

(1) Formed by blood corpuscles, both red and white, bursting into the tubules, and fixing themselves on to a hyaline cast.

(2) Appearance. Casts of various types showing undegenerated red cells in



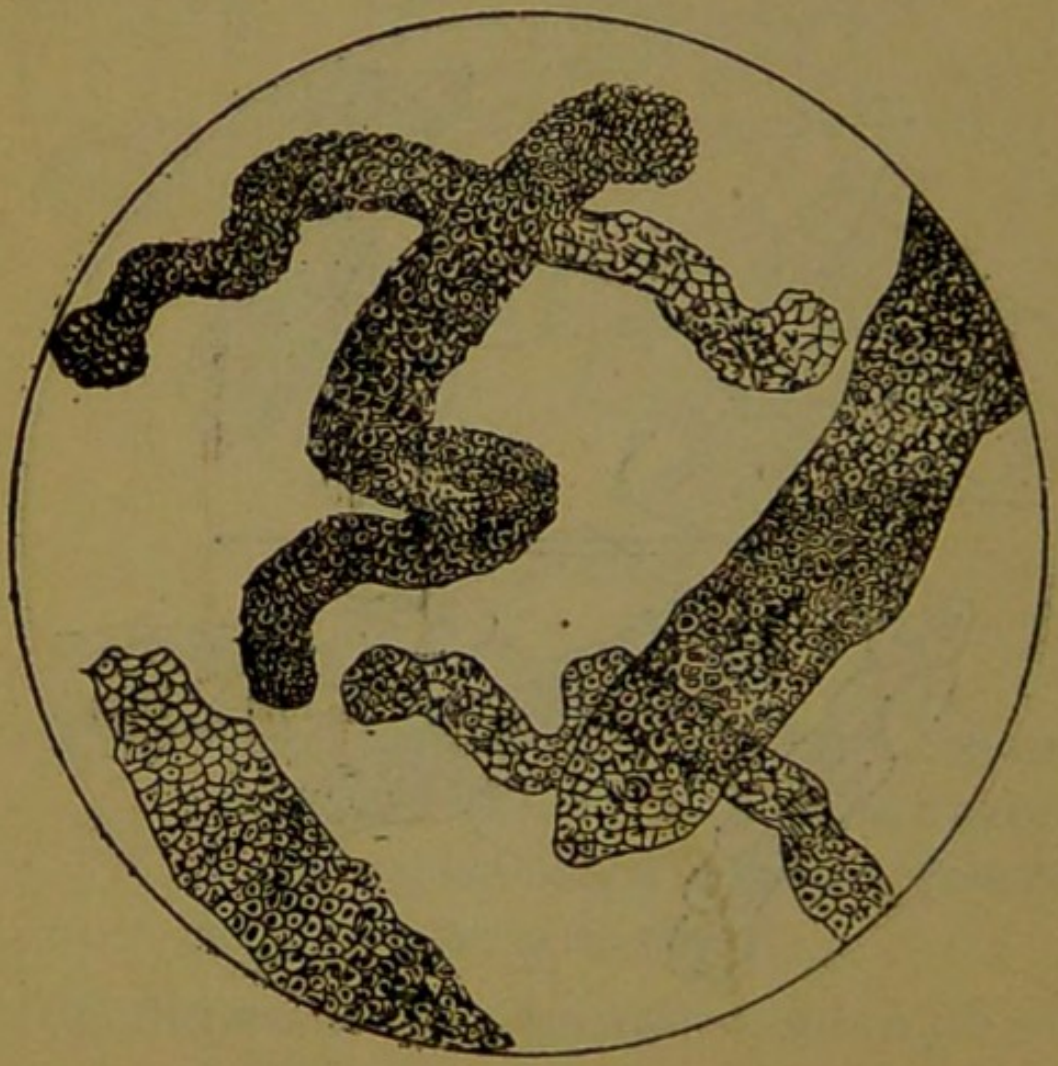
EPITHELIAL CASTS BLOOD CASTS

them ; the cast may be packed with red cells or only a few may be present.

(3) Significance. They are found typically in the early stages of acute nephritis, also in simple venous congestion due to heart disease.

GRANULAR CASTS.—

(1) Formed probably in the same way as epithelial casts, except that the cells have undergone some degenerative



GRANULAR CASTS

change before being shed, or while lying in the kidney tubules.

(2) Appearance. They appear coarsely granular, and the individual cells making up the cast are indistinguishable.

(3) Significance. They occur very frequently in various types of nephritis, but are most frequently found in chronic parenchymatous and in the later stages of acute nephritis.

FATTY CASTS.—

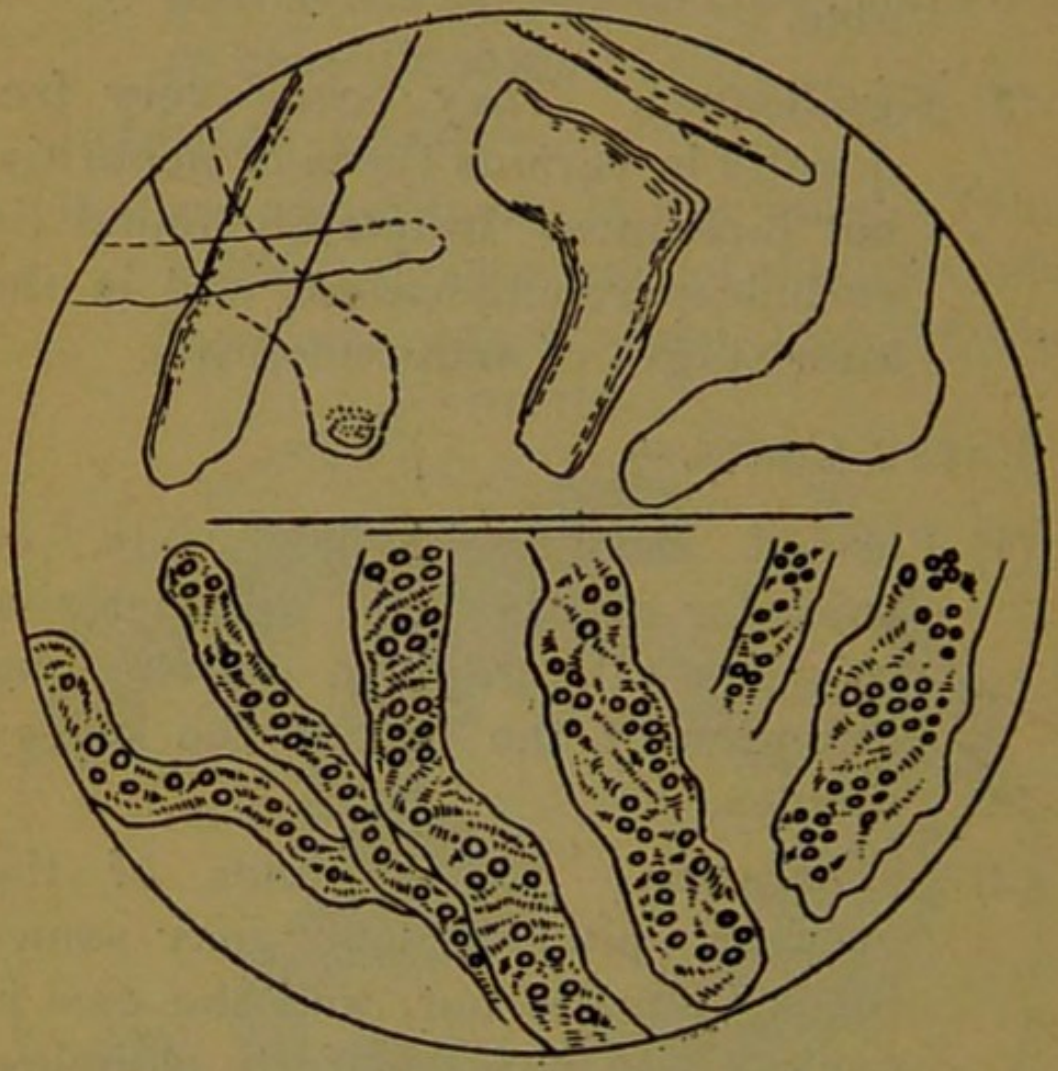
(1) Formed from epithelial casts, or granular casts by degenerative changes taking place due to long retention of the cast in the kidney tubules.

(2) Appearance. The outlines of the original epithelial cells may sometimes be made out, and the cast is packed with masses of fatty globules. The fatty globules may be indefinitely scattered through the cast without any preservation of cell outline.

(3) Significance. These casts represent a further degenerative change due to a slower elimination from the kidney.

WAXY CASTS.—(Colloid Casts)

(1) Formed by a degenerative change in the shed epithelial elements of the kidney tubules; this occurs when they are long retained.



FATTY CASTS WAXY CASTS

(2) Appearance. They are highly refractile with a sharp outline which often shows characteristic indentations in it. These casts do not usually give the amyloid reaction.

(3) Significance. They are not very common, but may be found in any case of advanced nephritis, and are not indicative of amyloid disease of the kidney.

ALBUMIN

Albumin is the most common abnormal protein in urine. It occurs chiefly as serum albumin, but usually there is a certain amount of serum globulin present also.

The significance of albumin in the urine varies greatly. It may be one of many indications that the kidneys are seriously damaged. It may, on the other hand, indicate a slight transient strain on the kidney due to excessive exercise, *e.g.*, the University Boat Race. The causes of albuminuria are legion, and cannot be detailed here.

When we speak of albumin being present in the urine, we mean serum albumin, which is the type most commonly found.

Certain precautions should be taken before testing any urine for albumin, viz.—

1. The urine must be fresh and clear.
2. The urine must be slightly, but distinctly, acid.
3. The specific gravity should not be too high, *e.g.*, 1015 or lower.

4. In doubtful cases it is safer to obtain an afternoon specimen, preferably after exercise, on which to carry out the tests.

The urine may be cleared by filtration, but in urines where bacteria swarm, mere filtration will not suffice to clear the urine. In such cases of bacteriuria, a little infusorial earth may be shaken up in the urine. This earth acts as a plug to the paper, allowing the filtrate to come through clear.

There are many tests for albumin. The more important will be described and the possible fallacies pointed out.

Heat and acetic acid test:— Fill a test tube three-quarters full of urine and heat the upper layers over a spirit flame. A cloud or haziness will form which may be due to—(1) Calcium phosphate, (2) Calcium carbonate, or (3) Albumin.

To differentiate, add drop by drop 3 per cent. acetic acid, which will dissolve the cloud if it be due to phosphates or carbonates; if due to the latter there will be effervescence with the disappearance of the cloud. If the cloud remains or becomes more intense, albumin is present.

In estimating whether a cloud is present or not, hold the test tube against a dark

background, and do not use a gas flame, which is apt to leave a deposit on the glass. Hydrochloric or nitric acid in small amounts may be used instead of acetic acid.

FALLACIES OF THE TEST :—

1. Nucleo-albumin in the urine would give a cloud on the addition of acetic acid. This source of error can be avoided by adding a saturated solution of sodium chloride to the urine in the proportion of $\frac{1}{5}$ NaCl solution to $\frac{4}{5}$ urine before boiling. This is often done as a routine procedure before heating the urine; also the error can be avoided by testing a specimen of cold urine, for acetic acid added to cold urine gives a cloud if nucleo-albumin be present.
2. If too much acetic acid be added, the cloud formed on heating might be dissolved up, therefore add the acetic acid drop by drop.
3. Patients taking resinous drugs, (*e.g.*, cubebs, turpentine, etc.) may give the reaction when acetic acid is added. This source of error can be controlled by cooling the test tube and adding alcohol, which dissolves up the resinous precipitate.

4. A precipitate might separate on cooling and redissolve on boiling if albumose were present.

Heller's nitric acid test:—This acid test is known as a contact test, because a white line appears at the junction of the acid and the albuminous urine. This is brought about by the transformation of albumin into acid albumin.

Pure concentrated nitric acid is poured into a wide test tube to the depth of about an inch. Urine is drawn up into a pipette, and slowly run down the side of the test tube to overlay the nitric acid. If albumin be present, a white line develops at the line of contact of the two fluids. This white line may require a few minutes to develop and even then be very faint. It is best seen against a black background with the eye on a level with the line of contact.

FALLACIES:—

1. Nitric acid may precipitate *urates* which would give a white ring. This would not occur in dilute urine. Heat dissolves the urates and causes the white ring to disappear.
2. Urine of patients who have been taking resins may give a cloud with this test. Heat dispels the cloud.

3. Nucleo-albumin may give a ring which is, however, diffusely spread *above* the line of contact.

4. Proto-albumose may also give a cloud, but this is dissolved by heating.

We therefore note that if a white ring develops at the line of contact, and is insoluble on heating or in a mild excess of the acid, we are dealing with a case of albuminuria.

Potassium ferrocyanide and acetic acid test.—To the urine in a test tube add a few drops of strong acetic acid. This will precipitate any nucleo-albumin, which may then be removed by filtration. A few drops of a 3 per cent. potassium ferrocyanide solution are added, and if a cloud appears it may be due to albumin or albumose. Now heat the tube, and if the cloud be due to albumose, it will disappear. The cloud due to albumin remains.

FALLACIES :—

1. The addition of too much ferrocyanide dissolves the cloud.

2. The other fallacies are eliminated in the performance of the test, viz., nucleo-albumin and albumose.

Tanret's mercuric chloride test.—Tanret's solution is made as follows:—

$HgCl_2$ – 1·35 grms. } dissolved in as little
KI – 3·32 grms. } water as possible.
Water – 50cc. } are added to the
Glacial acetic acid – 20cc. } above solution.

TEST.—Half a test tube is filled with urine and Tanret's solution poured in drop by drop. If albumin be present, a faint cloud develops as each drops falls into the urine. Two or three drops may require to be added before the hazy cloud forms.

FALLACIES:—

Nucleo-albumins. } all give a cloud, but this
Peptone. } disappears on heating.
Albumoses. }

Alkaloids, *e.g.*, quinine, etc., also give a cloud. This cloud also dissolves on heating.

N.B.—This test is considered by some to be too delicate for ordinary clinical work.

Salicyl-sulphonic acid test.—Add 10 to 20 drops of a 20 per cent. solution of salicyl-sulphonic acid to acid urine in a test tube. If albumin should be present, a turbidity or white precipitate develops depending on the quantity of albumin present.

FALLACIES.—In this method of testing,

albumoses and peptones are also "brought down," but on heating the tube they are re-dissolved.

There are many other tests for albumin, such as Johnson's picric acid test, Spiegler's test, and Jolles's modification of it, tests with orthophosphoric acid, tannic acid, etc.

The tests given above are sufficient for any urine examination. It is well always to perform at least two tests, and in doubtful cases three or four, taking special care to eliminate fallacies.

It may be remembered that at least one test for albumin can be carried out in any remote cottage, viz., boiling the urine and adding vinegar.

Quantitative Estimation of Albumin.—The most convenient "side-room" method of carrying out this estimation is still by means of Esbach's tube.

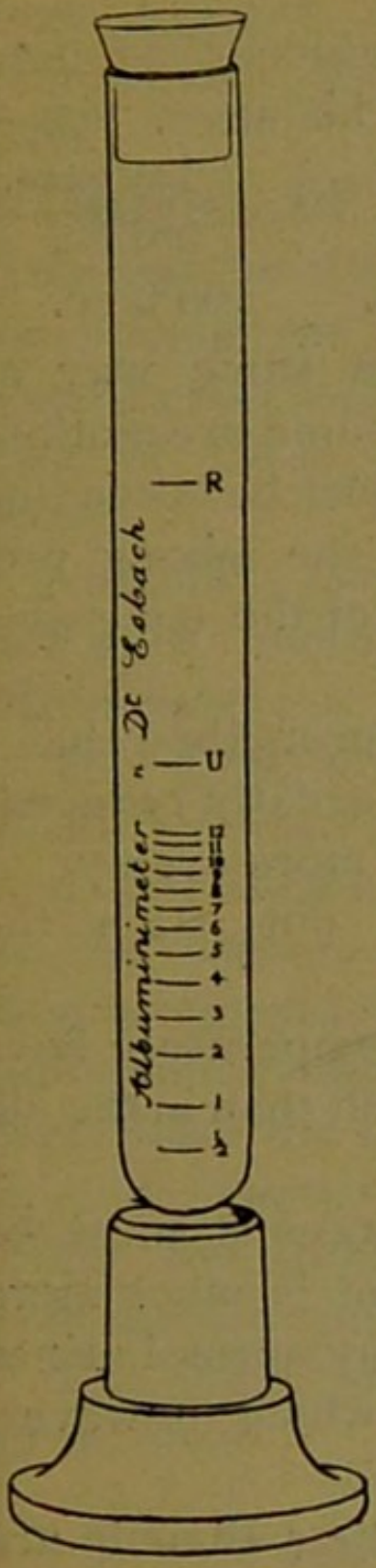
Esbach's albuminometer consists of a single thick test tube on which is marked a scale to enable one to read off the quantity of the precipitate formed in grammes per litre. Above this scale is a mark opposite the letter U, and a little above this is another mark opposite the letter R. The mouth of the tube is closed by a rubber cork.

Esbach's reagent consists of 20 grms. citric acid and 10 grams. picric acid dissolved in 1 litre of water.

PRECAUTIONS TO BE TAKEN BEFORE ESTIMATING.—

1. The urine must be acid.
2. The specific gravity of the urine should be about 1008, because the scale on the tube is calculated to read the albumin precipitate formed at the end of 24 hours in a urine of this density.
3. If there be more than ·4 per cent. to ·5 per cent. albumin, the reading will not be strictly accurate.
4. Picric acid precipitates out globulin as well as albumin. If, therefore, albumin alone is wanted, the globulin must be removed by the salting-out process.

METHOD OF PERFORMING THE TEST.—Acid urine with a specific gravity about 1008 is poured into the Esbach tube until it reaches the mark U. If the specific gravity be over 1012, the urine may be diluted with an equal bulk of water, and the final result must then be multiplied by 2.



The picric acid reagent is then added to the mark R. The rubber cork is now inserted, and the contents of the tube thoroughly mixed without causing bubbles to form. The tube is set aside in an upright position at ordinary room-temperature. The coagulum formed is allowed to settle for twenty-four hours and then read off on the scale. This scale shows the number of grms. of albumin per litre of urine. To bring the reading to grains per ounce, multiply the figure obtained by ·4.

EXAMPLE.—The top of the coagulum reaches the figure 3, *i.e.*, the urine contains 3 grms. dried albumin per 1000 cc. of urine, or ·3 per cent.

In grains per ounce it would read $3 \times \cdot 4 = 1 \cdot 2$ grains per ounce. It has been shown that this picric acid method has given rather

unreliable results at times.

Tsuchiya, therefore, introduced the following reagent which goes by his name, viz :—

Phosphotungstic acid	1·5 grm.
Conc. HCl	5·0 cc.
Ethyl alcohol 96 per cent.	100·0 cc.

It is used in exactly the same way as Esbach's reagent, and the same precautions must be observed. Care must be taken that no bubbles develop during the mixing process, as these cause parts of the coagulum to float.

It is claimed for this newer method that

(1) The average error is greatly reduced.
(2) The coagulum settles more evenly.
(3) The reagent does not stain the fingers.
(4) Differences of room-temperature have less effect on the result than in the old method.

Among all these advantages it must be remembered that the reagent is much more expensive ; also that in many normal urines a slight precipitate forms which, however, is negligible.

Other methods of estimating albumin are in vogue, such as precipitating all the albumin, filtering, drying, and weighing the filter which retains the albumin, Purdy's centri-

fuge method, etc. These are all more difficult and require special apparatus and skill.

REMOVAL OF ALBUMIN FROM URINE

This can be done with sufficient accuracy by boiling the urine, adding acetic acid, and filtering two or three times, or through two or three layers of moistened filter paper.

Another more elaborate and more accurate method is recommended by Hofmeister :—

The urine must be free from glucose. A 40 per cent. solution of sodium acetate and concentrated ferric chloride are added to the albuminous urine, the acetate solution being added first, and the iron solution added until a definite red colour is produced. Neutralise the urine and boil. Basic ferric acetate carries down all the albumin. Filter the mixture and the urine comes through albumin and iron free.

The other proteids which occur in the urine are—

(1) Albumose.
(2) Globulin.
(3) Nucleo-Albumin.
(4) Mucin.
(5) Blood.

Albumose.—This occurs as a pathological constituent in the urine, as a result of splitting of albumin. It may be found in the urine of patients suffering from some of the acute fevers, during the resolution stage of acute lobar pneumonia, acute yellow atrophy of the liver, in phosphorous poisoning, and leucocythæmia.

TEST.—Albumin if present must be removed.

The urine is strongly acidulated with concentrated acetic acid; to this is added phosphotungstic acid, and the mixture is left standing for some time. To get a positive reaction a milky cloud develops at once or in about 10 minutes. If negative, this test cannot be considered as very valuable. The precipitate may then be heated, and if it be due to albumose the cloud will clear and reappear on cooling.

The biuret test may be employed.—Albumin is removed and the filtrate concentrated. To 5 cc. of this concentrated filtrate 1 drop 2 per cent. $CuSO_4$ is added, and 5 cc. NaOH (10 per cent.) is also added. If albumose be present a pink colour is produced.

Globulin in urine may be removed by saturation with magnesium sulphate, or half

saturation with ammonium sulphate. The precipitate is removed by filtration, and the filtrate contains the serum albumin.

Bence-Jones proteid.—The substance occurs in the urine in cases of multiple myelomata. It may be detected thus :—

TEST.—Acid urine is slowly heated, and as the temperature rises above 50° C. a slight turbidity develops which becomes a sticky precipitate at about 60° C. As heating proceeds, the precipitate lessens in density, and when the urine boils, only a faint cloud remains or the urine may become quite clear. The precipitate returns on cooling.

Mucin may be considered a normal constitutent of urine, and when it is increased in amount, **nucleo-albumin** frequently accompanies it. The latter is derived from the breaking down of epithelium. Mucin is a glucoprotein and nucleo-albumin is a phosphoprotein.

Mucin and nucleo-albumin may be demonstrated by adding concentrated acetic acid in excess to very dilute urine, a precipitate forming in the cold. Globulins give the same reaction, but are soluble in excess of the acid.

The urine must be very dilute, to prevent the salts from dissolving the precipitate and the urates from remaining undissolved.

BLOOD IN THE URINE

Blood or its derivatives may occur in the urine under many pathological conditions. Blood in the urine may be classified under two groups, viz.—

(1) Hæmoglobinuria where we find the colouring matter of the red corpuscles alone, or with only a very few corpuscles present.

(2) Hæmaturia, where we find the normal constituents of the blood present in the urine.

Tests for Blood in the Urine

1. **Heller's Test.**—In normal urine we find a precipitate of phosphates and carbonates brought down by adding liquor potassæ and boiling the urine. If blood be also present, this precipitate is brownish in colour due to the hæmatin brought down with the alkalies. Normally the precipitate is white. The test is a delicate one.

FALLACY.—In an alkaline urine phosphates of the alkaline earths may have been already precipitated, which would cause failure of

the test, as KOH and heat would bring down no more.

Normal acid urine may be added to replace the salts already precipitated.

2. **Guaiac Test.**—To carry out this test it is advisable to prepare fresh guaiac solution each time. A knife point of powdered guaiac resin is dissolved in 5cc. of alcohol. To this is added an equal quantity of hydrogen peroxide, and the whole is thoroughly mixed. The guaiac mixture is then carefully layered over the suspected acid urine. If blood be present an opaque blue ring forms at the junction of the fluids. The test is very valuable if negative.

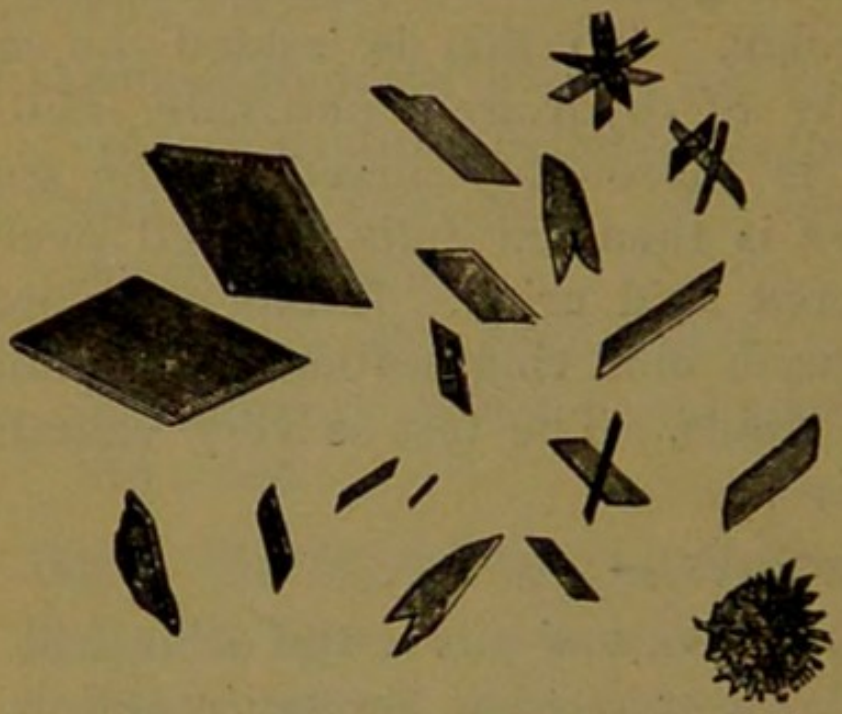
FALLACIES.—

(1) Saliva and pus in the urine will give a somewhat similar reaction, pus giving a greenish colour.

(2) A patient taking iodides will give a somewhat similar reaction, but in this case the blue takes longer to develop and diffuses more through the fluids.

3. **Hæmatin Test.**—(1) Precipitate the earthy phosphates from the urine placed in a test tube with caustic potash—heat

gently; (2) filter, and dry phosphates on a slide; (3) add a crystal of sodium chloride, and cover with a cover glass; (4) put a drop of glacial acetic acid on the slide, and allow it to come in contact with the phosphates and salt; (5) warm the slide. Allow to cool and examine under the microscope for hæmin crystals.



HÆMIN CRYSTALS

4. **Microscopic Test.**—The only absolute test for blood is by means of the microscope. A sample of the 24 hours urine is allowed to settle in a urine glass over night, and some of the deposit centrifuged. This deposit is examined under $\frac{1}{6}$ objective, and the presence or absence of blood corpuscles noted. The finding of even a few red blood

corpuscles is definite proof that blood is present.

If there be any doubt, the deposit may be dried and stained with Jenner, etc., and then examined. The red corpuscles will show up stained in this way, although they may be distorted in shape.

The microscope test is very valuable in certain cases where renal calculus is suspected, for the amount of blood passed may not be sufficient to give the chemical reactions. In hæmoglobinuria, on the other hand, the hæmoglobin is usually sufficient to give a positive chemical or spectroscopic reaction.

BILE IN THE URINE

Bile may occur in the urine in almost every type of jaundice as bile pigments or bile salts.

Bile Pigments.—Bilirubin is the only bile pigment which occurs in newly voided urine in pathological conditions. Biliverdin, bilifuschin, etc., develop from bilirubin only after the urine has stood for some time.

There are several tests for bile in the urine.

1. **Foam Test.**—Some urine is put in a test tube and vigorously shaken; if bile be

present the foam formed turns a distinctly yellowish colour. The test is a good rough one to employ.

The sediment of bilious urine is also stained yellow. Filter paper used in filtering iteric urine is similarly stained.

2. **Gmelin's Test.**—About one inch of yellow nitric acid is poured into a test tube. The suspected urine is now layered over the acid, and if bile be present a play of colours is visible at the junction of the fluids. The colour which must be distinctly visible is green, because if this is not seen, the test cannot be considered positive.

Rosenbach has modified the test, which he carries out in the following manner, viz.:—

The suspected urine is acidulated with a few drops of hydrochloric acid, and filtered two or three times through clean filter paper. This stained paper is now touched with a drop of yellow nitric acid, and, if bile be present, a play of colours spreads outwards from the drop. The green colour is due to the oxidation of bilirubin to biliverdin, and the test cannot be considered positive unless this green colour develops.

3. **Huppert's Test.**—Pour about 2 inches of suspected urine into a test tube. Make

the urine alkaline by adding a little sodium carbonate. A 10 per cent. solution of calcium chloride is now added drop by drop until no more precipitate forms. Filter. The precipitate is washed and then the filter paper is placed in an acid alcohol solution consisting of 5cc. conc. HCl and 95cc. alcohol. This mixture is heated and, if bile pigment be present, the alcoholic solution turns blue or green.

The test is useful when darkly pigmented urines are to be tested or when indican is present in the urine. It has succeeded at times when Gmelin's test has given a negative result.

4. Iodine Test.—The most delicate method of carrying out this test is to overlay the urine in a test tube with dilute tincture of iodine. At the junction of the fluids a green ring will appear at once, or in a few minutes, if bile be present.

N.B.—(1) If urine be high coloured it should be well diluted before carrying out the tests. (2) Some normal urines have been said to give this reaction.

When the colouring matters are small in quantity, the bile-pigment may be extracted from acid urine by the action of chloroform.

BILE ACIDS

Pettenkofer's Test.—This cannot be applied directly to the urine, which must first be prepared for the test.

PREPARATION OF URINE.—(1) Take 20 oz. of the urine and evaporate to a thick syrup, and then mix with ordinary alcohol. (2) Evaporate the alcoholic solution to dryness. (3) Dissolve the residue in absolute alcohol, and again evaporate. (4) Dissolve the residue in a little distilled water. (5) Add neutral and basic acetate of lead and set aside for twelve hours. (6) Collect the precipitate, and then treat it with a solution of sodium carbonate; filter. Pettenkofer's test can now be applied to the filtrate which contains the bile acids as sodium glycocholate and taurocholate.

PROCESS.—(1) Dissolve 2 or 3 grains of grape sugar in some water in a test tube, and add a small quantity of prepared solutions of bile acids. (2) Pour down the inside of the test tube about half a drachm of concentrated sulphuric acid. An intense purple colour will be seen at the junction of the two liquids. This test is rather tedious, and doubt exists as to its value when applied to urine.

Hay's test for the presence of bile acids.—The urine is cooled below 17° C., and a little placed in a watch glass. A few grains of powdered sulphur are thrown on the surface of this fluid, and if the urine contains bile salts, the sulphur sinks, owing to diminution of the surface tension; normally the sulphur will float on the surface. When bile acids are present in a small quantity only, the sulphur may not sink at once. The sinking process can be assisted by shaking the urine a little. This test is claimed by some to be more accurate than Pettenkofer's test.

SUGAR

Carbohydrates appear normally in the urine as glucose, animal gum, and isomaltose. Glucose ($C_6H_{12}O_6$) appears only as a trace, *e.g.*, ·001% – ·05%. This small quantity will give no reaction with the ordinary tests.

The tolerance of each individual to the assimilation of sugar is of the utmost importance from the therapeutic point of view. In doubtful cases it is, therefore, necessary to perform several tests, taking special care to eliminate all fallacies, before giving a definite opinion as to the presence or absence of sugar.

Before carrying out any of the tests for sugar, the urine may require some preliminary preparation, viz. :—

1. Dilute the urine.
2. Remove any albumin present.
3. Render the urine alkaline in most cases.

QUALITATIVE TESTS

Fehling's Test is probably the most frequently used of all the sugar tests, and when carefully performed, with a due regard to fallacies, is very valuable.

Two stock solutions are prepared and kept apart.

Solution A.

Copper sulphate . . .	34·64 grms.
Conc. H_2SO_4	1 drop
Distilled water to . . .	500 cc.

Solution B.

Sodium potassium tartrate .	173 grms.
Sodium hydrate . . .	125 ,,
Distilled water to . . .	500 cc.

The Rochelle salt is added to enable a maximum of the copper to remain in solution,

METHOD.—For each test equal quantities of solutions A and B are mixed. About an inch of urine is put in one test tube, and an equal quantity of the mixed solution in another. The contents of the two tubes are then boiled separately. The urine is run into the copper solution when they are both just off the boil. If glucose be present, a yellow or red precipitate forms immediately or gradually, due to the formation of cuprous oxide.

N.B.—In many non-saccharine urines a greenish-yellow colour may develop while performing the above test. This is not to be mistaken for sugar, but is due in many cases to the presence of substances like creatinin, uric acid, etc. It is noted in many gouty or constipated people.

FALLACIES.–Lactose, pentose, and lævulose give a positive Fehling's test. Urine from patients taking certain drugs, *e.g.*, salicin, morphine, antipyrin, and chloral hydrate, combined with glycuronic acid reduces the cupric hydroxide to cuprous hydroxide and cuprous oxide at boiling point or even a few degrees below that, but not as low as 80° C., hence the value of testing the urine when the solutions are completely "off the boil." This reduction brings suspicion to the mind of the

observer in that the reaction is atypical, the red granular deposit of cuprous oxide being absent.

If chloroform has been used to preserve the urine it may act as a reducing agent, and should be removed by boiling the urine.

A precipitate may appear on standing. This has no clinical significance.

2. **Almen-Nylander bismuth test.**—A stock solution of the reagent is made thus: —4 grms. Rochelle salt are dissolved in 100cc. of warm 10 per cent. sodium hydrate. This solution is saturated with bismuth subnitrate, about 2 grms. being required. The mixture is filtered and preserved in a dark bottle. This solution is cheap, and will remain good for years.

METHOD.—Take some dilute urine in a test tube, and add about $\frac{1}{10}$ volume of the reagent and boil. A black colour develops in the fluid, and is followed by a black precipitate of bismuth indicating the presence of sugar. If there be only a small quantity of sugar present, a greyish deposit will form as the white of the phosphates obscures the small black particles. The test is delicate, and reliable, if the fallacies are guarded against.

FALLACIES.—(1) A concentrated urine may give the reaction.

(2) Rhubarb and senna may give a reduction, but may be distinguished by noting a brownish colour which develops in the mixture before boiling.

(3) A positive reaction may follow the ingestion of certain other drugs, *e.g.*, salol, benzol, sulphonal, antipyrin, much quinine, etc.

(4) It is also positive after a person has eaten asparagus.

(3) **Phenylhydrazin test.**—This test used to be carried out with phenylhydrazin hydrochloride and sodium acetate, but recently Cippolina has simplified the method, the result being a very valuable test which is easily carried out. Albumin is removed from the urine, and a sample of diluted urine taken.

TEST.—To about an inch of urine in a test tube add about 5 drops of pure phenylhydrazin (the base—a liquid), and ·5 cc. of glacial acetic acid. Boil this mixture gently over a small flame for about a minute. Then add 4 or 5 drops of sodium hydrate—the mixture remaining acid—and boil the

whole again for a few seconds. The tube is now put aside to cool, and within twenty minutes (often immediately) typical phenyl-glucosazone crystals appear. They appear more rapidly in a low specific gravity urine, and can be confirmed by a high power of the microscope, appearing as golden yellow crystals arranged in sheaves.

FALLACIES.—Glycuronic compounds, pentose, maltose, lactose, etc., give a similar reaction, but the crystals formed differ in their melting points, and certain other reactions may assist in differentiating—pentose being the most likely source of error.

4. **Fermentation test.**—By means of this test we detect the presence or absence of a fermentable sugar.

The test may be carried out in an Einhorn fermentation tube or a Doremus ureometer. Enough dilute urine is taken to fill either of these tubes. A piece of *fresh* yeast is pounded up with the urine, and the mixture inserted into the tube in such a way that no air bubbles are present. The tube is placed in a warm temperature (*e.g.*, 15° C.—35° C.) and if sugar be present CO_2 begins to collect at the top of the tube. Two other control tubes must be set up, one containing normal

urine and yeast, the other a glucose solution and yeast, to determine that the yeast is active. After twelve hours the readings on the tubes are compared, and the presence or absence of a fermentable sugar determined by noting whether fermentation has taken place or not.

FALLACIES.—Urine containing lævulose and maltose ferment in the presence of yeast. If chloroform is used to preserve the urine it should be removed by boiling the urine. If there be only a trace of glucose present the CO_2 formed will be absorbed by the urine. In such a case fermentation should be carried out for a much longer period than usual, *e.g.*, 48 hours, and it will then be seen that any doubtful or definite reduction test will have disappeared.

There are other qualitative methods for determining the presence of sugar, but a sufficient number have been detailed.

The following table is taken from Emerson's text-book of clinical diagnosis, 3rd edition, and explains the most delicate methods of differentiating the different types of carbohydrates by commencing with the phenlyhydrazin test and then performing further tests.

The urine reduces Fehling's solution :—

- Gives crystals with phenylhydrazin directly in urine.
  - Melting point of crystals about 200° C.
    - Fermentation positive
      - Dextrorotatory — Glucose.
      - Lævorotatory — Levulose.
    - Fermentation negative — Lactose.
  - Melting point of crystals about 150° C.
    - Gives orcin reaction. — Pentoses.
    - Do not give orcin reaction. — Isomaltose.
- Gives crystals with phenylhydrazin only after the urine has been warmed with dilute sulphuric acid. — Paired glycuronic acid compounds.

QUANTITATIVE ESTIMATION OF GLUCOSE

Many methods are at present in use to calculate the daily output of sugar. Four methods will be described.

1. Benedict's Second Method of Estimating Sugar (*Jour. Biol. Chem.*, 1911).

In 1907 Benedict described a method of estimating the quantity of sugar by titrating the urine against an alkaline copper solution, made with a carbonate instead of a hydroxide, in which was dissolved potassium sulphocyanide. On boiling this solution and adding sugary urine, a reduction takes place forming a white precipitate of cuprous sulphocyanide, thus doing away with the difficulties in determining the end point when all the copper is reduced.

This process necessitates the preparation of three stock solutions.

Benedict therefore introduced his second method where only one stock solution, which will keep for a long time, is used.

The principle on which the method is based is to produce a precipitate of white copper sulphocyanate, and to prevent the formation of any red cuprous oxide by adding ferrocyanide of potassium. This

latter precipitate might occur in the presence of impurities, *e.g.*, chloroform.

This method is much easier to perform than Fehling's method, which has recently been described as "one of the most difficult of quantitative determinations in clinical chemistry."

The following solution constitutes the reagent :—

Crystallised copper sulphate,	18·0 grms.
Anhydrous sodium carbonate,	100·0 ,,
Sodium citrate,	200·0 ,,
Potassium sulphocyanate,	125·0 ,,
Five per cent. potassium ferrocyanide solution,	5·0 cc.
Distilled water to	1000·0 ,,

Benedict describes the preparation of this solution and the procedure in making the estimation thus :—

"With the aid of heat dissolve the citrate, carbonate, and sulphocyanate in enough water to make 800cc. of the mixture and filter. Dissolve the copper sulphate separately in about 100cc. of water and pour the solution slowly into the liquid, with constant stirring. Add the ferrocyanide solution, cool, and dilute to exactly the one litre. *Of the various constituents only the copper salt need be weighed with exactness.* 25cc. of

the reagent are reduced by ·05 grm. of glucose."

The estimation is made by using urine diluted 1 in 10 from a burette in the following way :—"Measure 25cc. of the reagent into a porcelain evaporation dish, and add 10-20 grms. of crystallised sodium carbonate (or half the weight of the anhydrous salt) and a very small quantity of powdered pumice stone. Heat the mixture to vigorous boiling over a free flame, and run in the sugar solution (urine) quite rapidly until a heavy white precipitate is produced and the blue colour of the solution begins to diminish perceptibly. From this point the sugar solution (urine) is run in more and more slowly with constant vigorous boiling until the disappearance of the last trace of blue colour, which makes the end point."

"The additional carbonate is added prior to the titration in order to provide sufficient alkalinity to ensure the production of a sharp end point."

The percentage of sugar may now be calculated by dividing ·05 by the number of cc. of diluted urine used and multiplying the result by 1000.

To turn the result into grains per ounce

multiply the percentage reading by the figure 4·375.

2. **Carwardine's Saccharometer Method.**—A quick and easy way of estimating the quantity of sugar may be done by Carwardine's Saccharometer where Fehling's solution is used. The solution is boiled in a test tube which can be held up to the light, and the presence or absence of the blue colour easily determined. The instrument is bought ready made along with full directions for its use.

It consists of:—

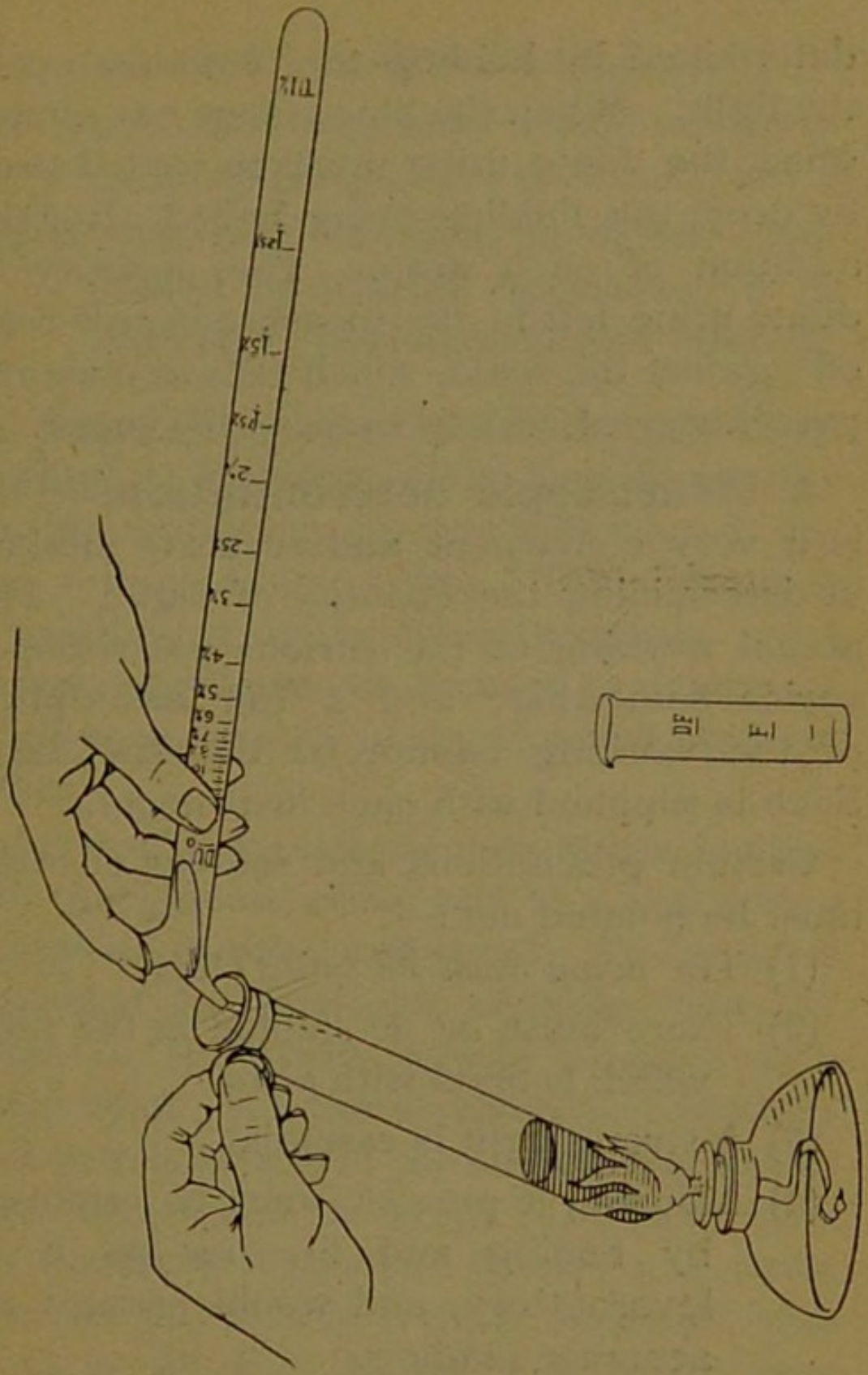
(1) A large test tube with a small metal handle.

(2) A measure for the Fehling's solution.

(3) A special type of burette graduated in percentages and marked off for measuring and diluting the urine.

METHOD OF USING.—Fill the measure with Fehling's solution to the mark F. Add water to the mark DF. Fill the graduated tube with urine to the mark U, and add water to the mark DU. Mix thoroughly.

Transfer the Fehling's solution to the test tube and boil it. Then add diluted urine slowly till the blue colour has been completely discharged. This can easily be



CARWARDINE'S SACCHAROMETER
Test Tube with Fehling's Solution.
Special Measure,
Graduated Burette.

determined by holding the test tube up to the light. When the blue colour has almost gone, the dilute urine must be added drop by drop, the Fehling being boiled after the addition of each drop. The quantity of dilute urine left in the measure is now read off against the scale, which tells at once the percentage of urine present in the sugar.

3. **Polariscopic determination.**—This is a very convenient and accurate method of determining the quantity of sugar. The actual working of the various polariscopes varies somewhat, and a full description of the working cannot be detailed here. Such is supplied with each instrument.

Certain precautions and sources of error must be pointed out :—

(1) The urine must be quite clear.

(2) There must be no bubble in the tube which is filled with urine.

(3) A strong light is essential.

(4) Albumin, if present, must be removed by boiling and filtering as it is levorotatory, and would prevent an accurate reading.

(5) The urine must be acid, because in alkaline media some of the glucose might turn into levulose.

(6) β-oxybutyric acid is levorotatory.

(7) Levulose in the urine would cause the reading for glucose to be too low.

(8) Preliminary clarification of the urine should be effected by shaking it up with basic lead acetate and filtering.

4. Specific gravity fermentation method of Roberts.—The specific gravity of urine containing glucose falls when it has been fermented by yeast. This difference of specific gravity between fermented and unfermented urine can be used to determine roughly the quantity of sugar which a patient passes.

METHOD.—A twelve-ounce bottle is filled with the glucose urine, and to it are added several small masses of yeast. The yeast is emulsified by thorough shaking, and the mouth of the bottle lightly plugged with cotton wool. Another twelve-ounce bottle is filled with a sample of the same urine, but no yeast added, and the bottle is corked. The two bottles are now put aside in a warm place for 24 hours at a temperature of from 25° C. to 35° C. At the end of this time fermentation should be complete, but it is safer to test the fermented urine to make certain of this fact.

The specific gravity of the two specimens is now taken under the same conditions of temperature. The number of degrees of difference between the fermented and un fermented urine represents the number of grains of sugar per ounce of urine the patient is passing. To express the result as a percentage multiply the difference by the factor ·23.

EXAMPLE.—The specific gravity of the unfermented sample is 1030 and that of the fermented sample 1010, difference 20. The urine, therefore, contains 20 grains of sugar per ounce, or 4·6 per cent.

LEVULOSE ($C_6H_{12}O_6$)

Levulose is a sugar which sometimes occurs in urine, and is usually associated with dextrose.

TESTS.—It reduces Fehling's solution, ferments with yeast, forms an osazone with phenylhydrazin, and rotates the plane of polarised light to the left nearly twice as strongly as dextrose rotates it to the right.

DIFFERENTIATING TEST FOR LEVULOSE.—Place two inches of urine in a test tube, add half-inch of dilute hydrochloric acid and a knife point of resorcin. Heat the mixture. If levulose be present, the mixture becomes

red and deposits a dark red precipitate.

Glucose, lactose, and pentose do not give this test.

LACTOSE ($C_{12}H_{22}O_{11},H_2O$)

Lactose occurs at times in the urine of lactating women in whose lacteal glands stasis has occurred. Patients who have been on a milk diet for a prolonged period may pass lactose in the urine.

The reduction tests for sugar are positive, but more slowly than those for glucose. Polarised light is rotated to the right.

TEST FOR LACTOSE.—Boil urine with excess of sugar of lead for three to four minutes. The solution becomes a yellowish-brown. Add ammonia to this hot fluid until the precipitate ceases to dissolve. If lactose be present, an intense brick-red fluid is obtained. Put this aside to settle, and a copper-red precipitate forms below a clear fluid. Glucose gives a red solution from which a yellow precipitate settles out.

PENTOSE

Pentose is present chiefly in vegetable tissues, and some individuals have the peculiarity of passing pentose after the ingestion of certain fruits such as pears,

apples, plums, cherries, and certain vegetables, *e.g.*, turnips. The condition has also been noted after drinking beer. The condition is not connected with diabetes mellitus.

Pentose reduces copper and bismuth slowly and incompletely. It does not ferment yeast. It may be suspected when the reduction tests are typical, when no fermentation takes place, and when there is no rotation with polarised light.

TESTS—THE PHLOROGLUCIN TEST.—Dissolve a knife point of phloroglucin in 1 inch of conc. hydrochloric acid in a test tube, and divide the mixture between two tubes. To one tube add 10 drops of the urine to be tested, and to the other add 10 drops of normal urine. Heat the tubes gradually in a water bath. The tube containing pentose turns reddish, the other remaining unchanged.

The test may be carried further by cooling the contents of the tube, extracting with amyl alcohol, and examining the extract by means of the spectroscope, which will show a band between D and E if pentose be present.

FALLACIES.—(1) Glycuronic acid gives the same reaction, but not short of boiling, and the same appearance with the spectroscope.

(2) Lactose gives the colour reaction, but not the spectrum band.

THE ORCIN TEST.—Into a test tube pour equal quantities of urine and concentrated hydrochloric acid. To this mixture add a knife point of orcin and boil the whole gently. A positive reaction is shown when the fluid turns dark greenish, later becoming turbid due to precipitation taking place.

The test tube should now be cooled till its contents are tepid, and then extracted by adding amyl alcohol. A positive reaction gives an olive green colour to the alcoholic extract.

FALLACY.—This test is given by paired glycuronates, but "comes out" more slowly than with the phloroglucin.

GLYCURONIC ACID ($CHO(CHOH)_4COOH$)

Glycuronic acid in not voided, as such, in the urine. It is excreted like sulphuric acid in combination with phenol, skatol, and indol, forming a potassium glycuronate of these substances. Certain drugs also have the effect, when administered in large quantities, of forming glycuronic compounds, *e.g.*, chloral, chloroform, morphine, camphor, and turpentine.

CAMPHOR AND TURPENTINE TESTS.—Most

of these glycuronates reduce Fehling's solution, some, however, only after cleavage by prolonged boiling with a dilute acid. They rotate the plane of polarised light to the left, but on cleavage as above, the glycuronic acid produced is dextro-rotatory.

The glycuronates do not give the Cippolina phenylhydrazin test, but if glycuronic acid be liberated the test becomes positive.

With the phloroglucin test no reaction takes place short of boiling, if, therefore, a urine fails to give these tests at 96° C. but gives them when boiled for three minutes, it may be assumed that glycuronates are present and are split up by boiling into glycuronic acid. This, and examination by the polarimeter, distinguish them from pentose. The failure to ferment distinguishes them from glucose.

NEUMANN'S TEST is useful in comparing several carbohydrate bodies. It is performed as follows:—To a quarter of an inch of urine in a test tube add an inch of glacial acetic acid and a few drops of a 5 per cent. solution of orcin in alcohol. The mixture is boiled, and 1 per cent. sulphuric acid is dropped in, the tube being shaken after every few drops till a permanent colour results.

Glucose gives a brownish-red colour.

Levulose gives a yellow-brown colour.

Arabinose gives a violet-red colour.

Glycuronic acid gives a green or greenish-blue colour.

ORGANIC ACIDS, ETC., IN THE URINE

The source of origin of acetone, diacetic acid and β-oxybutyric acid in the body has for long been doubted, but it seems fairly certain that they develop when fat is broken up in an abnormal way during its absorption into the body. Normally, fat is resolved into its end products, carbon dioxide and water, with the production of heat. An abnormal process of fat-splitting occurs when the tissues of the body are unable to obtain sugar from the blood.

This state of affairs occurs most markedly in diabetes mellitus. It has also been noticed in a minor degree in cases of starvation, toxic vomiting, cyclic vomiting, and delayed chloroform poisoning.

It is to be noted that β-oxybutyric acid, diacetic acid, and acetone are not in themselves poisonous, except in enormous doses. The severe symptoms which develop when these substances occur in large quantity in the urine, are due to the diminished alkalinity

of the blood brought about by the presence of these abnormal acids in the plasma. A condition, therefore, of "acidosis" is said to develop. This develops as a gradual break-down of the defences of the body against the inroads of these abnormal acids. When the abnormal acids begin to develop, the alkalies of the tissues are called upon to neutralise them, *e.g.*, potassium and sodium, then large quantities of ammonia are formed which unite with the abnormal acids, thus diminishing the formation of urea.

This causes the output of ammonia-nitrogen to rise at the expense of the urea-nitrogen. Ultimately, the amount of ammonia formed gives out, and then the alkalinity of the blood diminishes giving rise to toxic symptoms.

β-OXYBUTYRIC ACID ($CH_3CHOHCH_2COOH$)

There is no very simple test for β-oxybutyric acid, but the following test of Stuart-Hart is fairly simple :—

TEST.—Mix 20cc. of suspected urine, 20cc. of water, and a few drops of acetic acid. Boil this mixture till its volume is reduced to about 10cc. This drives off the acetone and diacetic acid. Add water to restore the bulk to 20cc. Divide this equally between two test tubes A and B. To A add 1cc.

hydrogen peroxide, warm gently, but do not boil, for a minute. Cool. Then add ½ cc. of glacial acetic acid to each tube and a few drops of a fresh solution of sodium nitroprusside. Mix the contents of each tube and overlay the mixture in each tube with ammonium hydroxide. Put the tubes aside to stand for four to five hours. At the end of this time compare the tubes, and, if the reaction be positive, tube A to which the hydrogen peroxide was added will show a purplish-red contact ring. This is due to the gradual oxidation of the β-oxybutyric acid to acetone by the hydrogen peroxide. If the tube is shaken up, the difference in colour will be seen in the fluid. This colour intensifies on standing 20 minutes or so. The control tube B is necessary in order to see whether the acetone and diacetic acid in the urine has been completely driven off in the earlier stages of the test.

The reaction is not interfered with by the presence of sugar.

DIACETIC ACID (CH_3COCH_2COOH)

This acid is the precursor of acetone, and it is of the utmost importance to test urine for its presence or absence in cases of acidosis, diabetes, etc.

PRECAUTIONS BEFORE TESTING :—

1. Urine must be tested shortly after it is voided. If this precaution be neglected, then the acid changes to acetone and its presence is missed.
2. If the urine has to be kept, add toluol to the specimen.

TEST.—Half fill a test tube with the suspected urine. Add drop by drop 10 per cent. ferric chloride solution, a deposit of iron phosphate will form. Filter. To the filtrate add a few more drops of iron solution. If diacetic acid be present a dark reddish-brown colour develops. The fluid in the tube is now divided, one half boiled, the other used as a control. If the colour reaction be due to diacetic acid, heating will decompose the acid and the colour will quickly diminish in intensity and ultimately disappear. The same will happen if the tube stands for some hours.

FALLACIES. —Patients taking drugs such as salicylic acid, salol, aceto-salicylic acid, phenacetin, etc., will give the same Bordeaux red reaction, but on heating and boiling the colour does not fade, no change takes place if the tube stands for many hours. If much diacetic acid be present, this change in colour on boiling does not take place.

ACETONE (C_3H_6O)

This substance may appear in diabetic urine, and in the urine of people suffering from acidosis where diacetic acid has been present.

LANGE'S TEST.—A test tube is half filled with the suspected diluted urine. To this is added a few drops of glacial acetic acid. A freshly prepared solution of sodium nitro-prusside is made up (4 grms. to the ounce). Add a few drops of this solution and layer ammonia above the urine. The presence of acetone gives an intense violet ring at the line of contact.

This test is claimed to be more delicate than Legal's original one, and the reaction has not the fallacy of being brought about by alcohol or aldehyde.

LIEBEN'S TEST is also very delicate, and considered by some authorities to be the most delicate and most reliable.

It is carried out as follows:—The urine should be distilled, although this is not absolutely essential. 5cc. of the distillate are placed in a test tube. To this are added a few drops of KOH and a little Lugol's iodine solution. The mixture is warmed and, if much acetone be present,

an immediate precipitate of yellow iodoform crystals is observed. One can also detect the smell of iodoform.

If only a small quantity of acetone be present (*e.g.*, ·001 grm.) the tube must be laid aside for some hours, and the crystals allowed to separate out. The deposit may be examined under the microscope to detect the presence of the typical hexagonal plates of iodoform crystals.

THE DIAZO REACTION

The diazo reaction never takes place in normal urine, but is produced by the presence of pathological constituents, as yet unknown, in the urine of patients suffering from certain diseases, *e.g.*, typhoid, acute tuberculosis, etc.

METHOD OF PROCEDURE :—

REAGENTS.—

Solution No. 1.

·5 per cent. watery solution of sodium nitrite. (This does not keep well.)

Solution No. 2.

Sulphanilic acid,	1 grm.
Conc. HCl,	5 cc.
Aq. dest. ad,	100 cc.

Solution No. 3.

Ammonia.

Many ways of carrying out the test have been suggested. The following, recommended by Dr Ker of the Edinburgh Fever Hospital, is simple and very satisfactory :— A third of an ordinary test tube is filled with urine, and an equal quantity of solution No. 2 added. Add to this one or at most two drops of solution No. 1. The tube is then well shaken to obtain a good foam. Render alkaline by allowing ammonia to trickle down the side of the tube, and if the reaction be positive the foam shows a beautiful pink colour, and the body of the urine is crimson. In a normal urine the froth is yellow, and the body of the urine a yellowish-red colour. The pink colour in the foam will persist for a few minutes at least, and is considered to be the diagnostic point. The colour gradually fades away. It is not to be mistaken for a reddish-brown which develops at times and disappears almost at once when the tube is set aside.

DRUGS IN THE URINE

It is frequently necessary to test the urine to determine if certain drugs are excreted or not. Further, it is well to keep in mind certain drug reactions, because they may be mistaken for other reactions caused

by some pathological constituent in the urine, *e.g.*, the ferric chloride test for diacetic acid is closely simulated by the reaction of antipyrin in the urine.

Antipyrin.—Urine containing this drug has a light-greenish appearance in reflected light, and a slight reddish colour when the light is transmitted. On the addition of liq. ferri perchlor. a reddish-brown colouration is gradually produced, which does not disappear with heat as it would if the reaction were due to diacetic acid.

Carbolic Acid (Phenol). This drug is excreted in the urine in combination with the sulphates. A urine of this type develops a dark greenish-black colour on standing for some time. The suspected urine is distilled over, and to the distillate is added 5 per cent. H_2SO_4. To this is next added ferric chloride which will give a more or less violet solution.

Balsam of Copaiba.—This drug in the urine will give a positive Trommer test and a negative Nylander, *i.e.*, it reduces copper, but not bismuth.

To the urine add drop by drop dilute hydrochloric acid, and a resinous precipitate will develop which gives a reddish-violet appearance.

Iodine.—This drug may be detected in the urine by adding 1cc. of dilute HNO_3, and ·5cc. chloroform. The mixture is shaken gently, and after settling, the chloroform is seen at the bottom of the tube coloured rose-pink.

Remember that Iodides give a blue colouration with tinct. guaiac. and ozonic ether.

Rhubarb, Senna, Rhamnus group, etc.—The urine passed is yellowish-brown in colour, and by the addition of liq. potassæ a more or less distinct red colour develops.

This red colouring matter will not dissolve in amyl alcohol.

Salicylates and Salol.—Urines containing the derivatives of these drugs will turn a violet colour if ferric chloride be added drop by drop to the specimen. Heating will not cause the colour to disappear as it would if the reaction were due to diacetic acid.

Santonin.—This drug gives the urine a peculiar saffron-yellow or greenish colour. This colour is turned to rose-pink by the addition of liq. potassæ. This colouring matter is dissolved up by the addition of amyl alcohol and shaking the mixture.

Urotropine (Hexamethylenetetramine).—This drug is broken up in the body and eliminated as formalin. It may be tested for by adding liq. potassæ and a few grains of resorcin to the suspected urine. A red colour develops at once, or in a few minutes.

Lead.—There is no very simple method of detecting this drug in the urine. A strip of shining magnesium free from lead may be placed in the urine, and some hours later removed. A deposit of lead will have formed which must be tested in a chemical laboratory. The lead is dissolved off by nitric acid.

BACTERIOLOGICAL EXAMINATION OF THE URINE

The examination of the urine for the presence of bacteria is a procedure from which much valuable information may be gained. In general practice it is frequently neglected. The method of collecting the urine is simple. In the case of a female patient, a nurse passes a catheter and collects the urine in a sterile glass bottle.

In the case of a male, the meatus, etc., are thoroughly cleansed, a catheter passed, and the urine drawn off into a sterile bottle. If

this method is objected to by the patient, he may thoroughly cleanse the meatus and glans penis. He is then directed to make water and allow the first portion of the stream to flow away. The remainder of the stream is passed into a sterile bottle.

If a film preparation alone is required, catheterisation is not necessary, but when cultures are to be made it is necessary.

The urine is centrifuged. A drop of the deposit is examined to note the presence or absence of motile organisms, at the same time note may be taken of other elements in the deposit.

Film preparations are now made in the usual way on microscope slides.

These films are stained with :—

(1) Carbol-thionin and (2) Gram's stain using dilute fuchsin as a counter stain. The staphylococci and M. ureæ retain the purple tint while gonococci lose it and appear pink.

The colon bacillus is the most frequent organism present in urine, and the method of investigating for its presence is as follows :—

(1) Pour out 5cc. of the urine into a broth-culture tube.

(2) Incubate at 37° C. for 24 hours.
(3) If B. coli are present the broth turns turbid and the organism can be demonstrated by the microscope.
(4) From this broth-culture take a loopful of fluid and make streaks on an agar petri dish.
(5) A series of strokes is now made on a petri plate of M'Conkey's medium with the same loopful of fluid.
(6) These cultures are incubated for 18-20 hours.

Colonies of the cocci, saprophytes, etc., are found on the agar, while the M'Conkey medium plate shows only cultures of B. coli. The colonies grown are subcultured through the various tests for B. coli described in bacteriological books.

Detection of tubercle bacilli in the urine.—Urine passed during twenty-four hours must be taken and allowed to sediment. This sediment is treated with 1 in 20 carbolic acid, and films are made and stained in the usual way (see p. 157). It is very important to remember the alcohol stage of the decolorising in order to be certain that the possibility of the smegma bacillus has been eliminated. It is further necessary to remember that a red-stained organism

seen against a pink field is probably a B. coli in an incompletely decolorised field. The tubercle bacillus will stain red, but must be seen against a blue background.

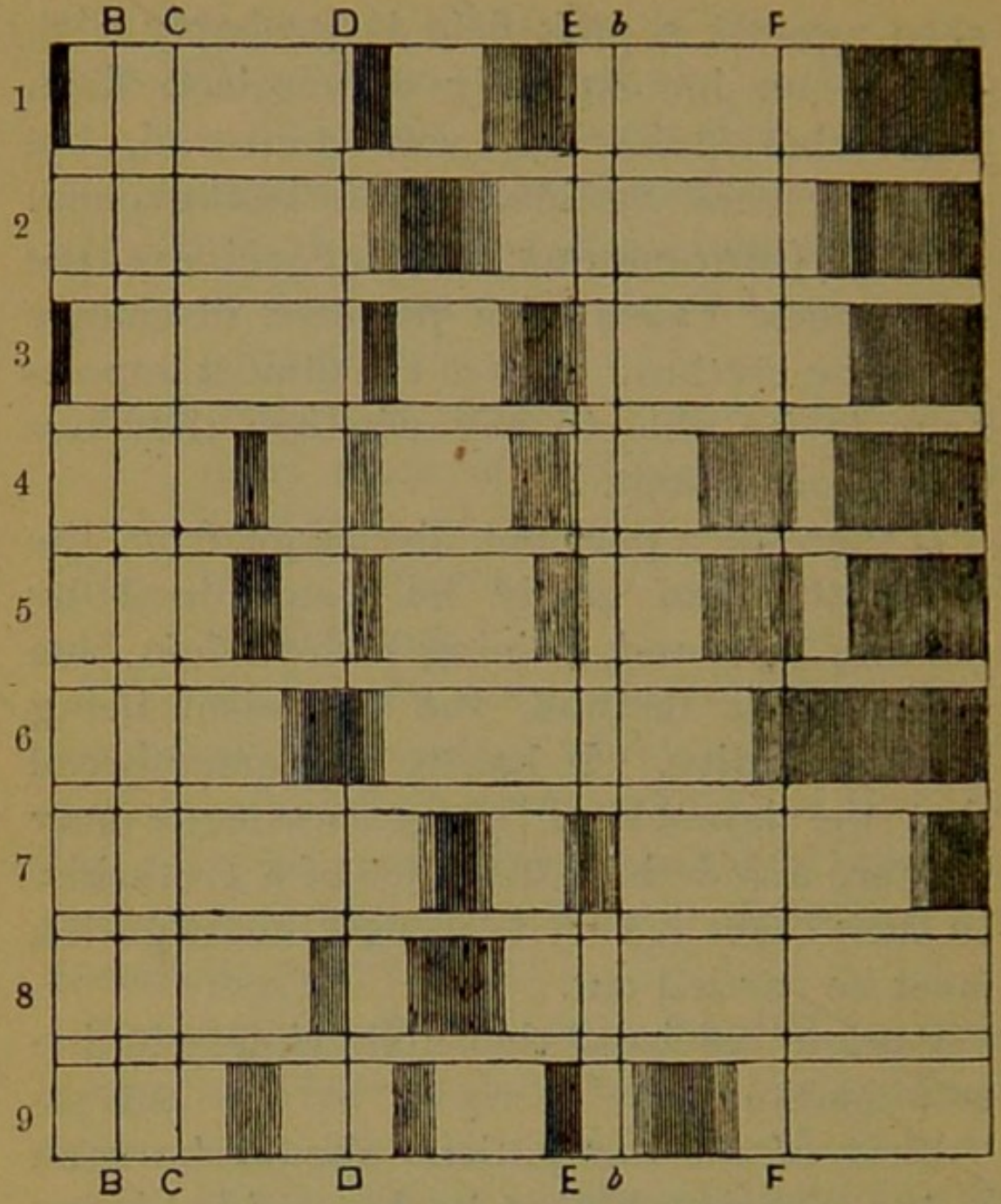
The Gonococcus.—Gonococci are the commonest cause of a purulent discharge from the urethra. It is of the utmost importance to be able to say whether they are absent or present.

If there be a purulent discharge from the penis this can easily be determined by making films and staining with thionin blue and Gram's method, the organism being Gram-negative. It has to be remembered that the latency of the gonococcus may obscure any definite evidence of a urethritis. In such cases a very thorough investigation must be carried out.

Panton advises the following routine examination :—

First of all the patient should be seen in the morning before he has voided urine to see if any slight discharge is present at the meatus. If a discharge is obtainable, films must be made.

Secondly, the patient passes urine and the deposit is centrifuged and pus cells sought for. If they are present, a careful search is made for the organism.



SPECTRA OF HÆMOGLOBIN AND DERIVATIVES

B, Oxygen line; *D*, Sodium line; *C* and *F*, Hydrogen lines; *b*, Magnesium line; 1, Spectrum of oxy-hæmoglobin; 2, Reduced hæmoglobin; 3, Carbon monoxide hæmoglobin; 4, Methæmoglobin (in acid solution); 5, Acid-hæmatin (in ethereal solution); 6, Alkaline hæmatin; 7, Hæmochromogen; 8, Hæmatoporphyrin (in acid solution); 9, Hæmatoporphyrin (in alkaline solution).

Thirdly, irrigate the anterior urethra with sterile water, and with the patient in the knee-elbow position, massage the prostate vigorously in order to express some prostatic fluid. This may bring away pus cells in which the gonococcus may be demonstrated.

If all these procedures prove negative, a further examination should be made at a later date. On this occasion, however, a large sound should be passed, and the urethra should be syringed with a silver nitrate solution. These procedures will stimulate inflammatory cells which must be examined for the gonococcus.

If these two complete examinations prove negative, we may say the patient is as free from infection as medical skill can ascertain. Examination for Typhoid bacilli, staphlococci, spirochætæ pallidæ may have to be made.

THE BLOOD

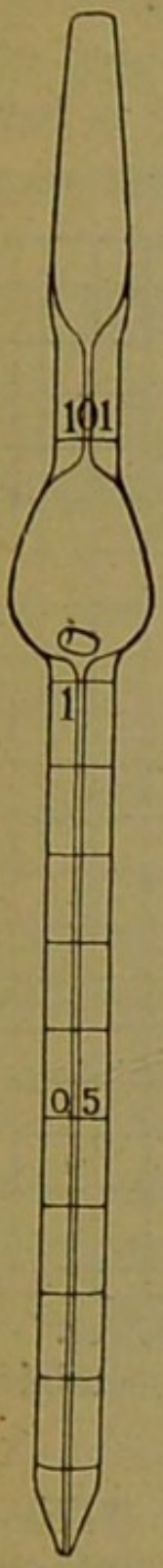
The routine methods employed in making a blood examination will be described.

These include :—

1. The enumeration of the red blood corpuscles.
2. The enumeration of the white blood corpuscles.
3. The estimation of the haemoglobin.
4. The colour index.
5. Preparation of a fresh and of a stained blood-film.
6. Glycogen reaction, in some cases.
7. The coagulability of the blood, in special cases.

Enumeration of the Blood Corpuscles.—Instruments and reagents required—(1) Microscope with mechanical stage; (2) Glass slide counting chamber (*e.g.*, Thoma-Zeiss); (3) Specially prepared pipettes with rubber tubing attached; (4) Diluting fluids; (5) Pricker; (6) Soft rag; (7) Ether or methylated spirits.

Procedure in Counting Red Blood Corpuscles.—The pipette used for this purpose has a very fine bore leading up into

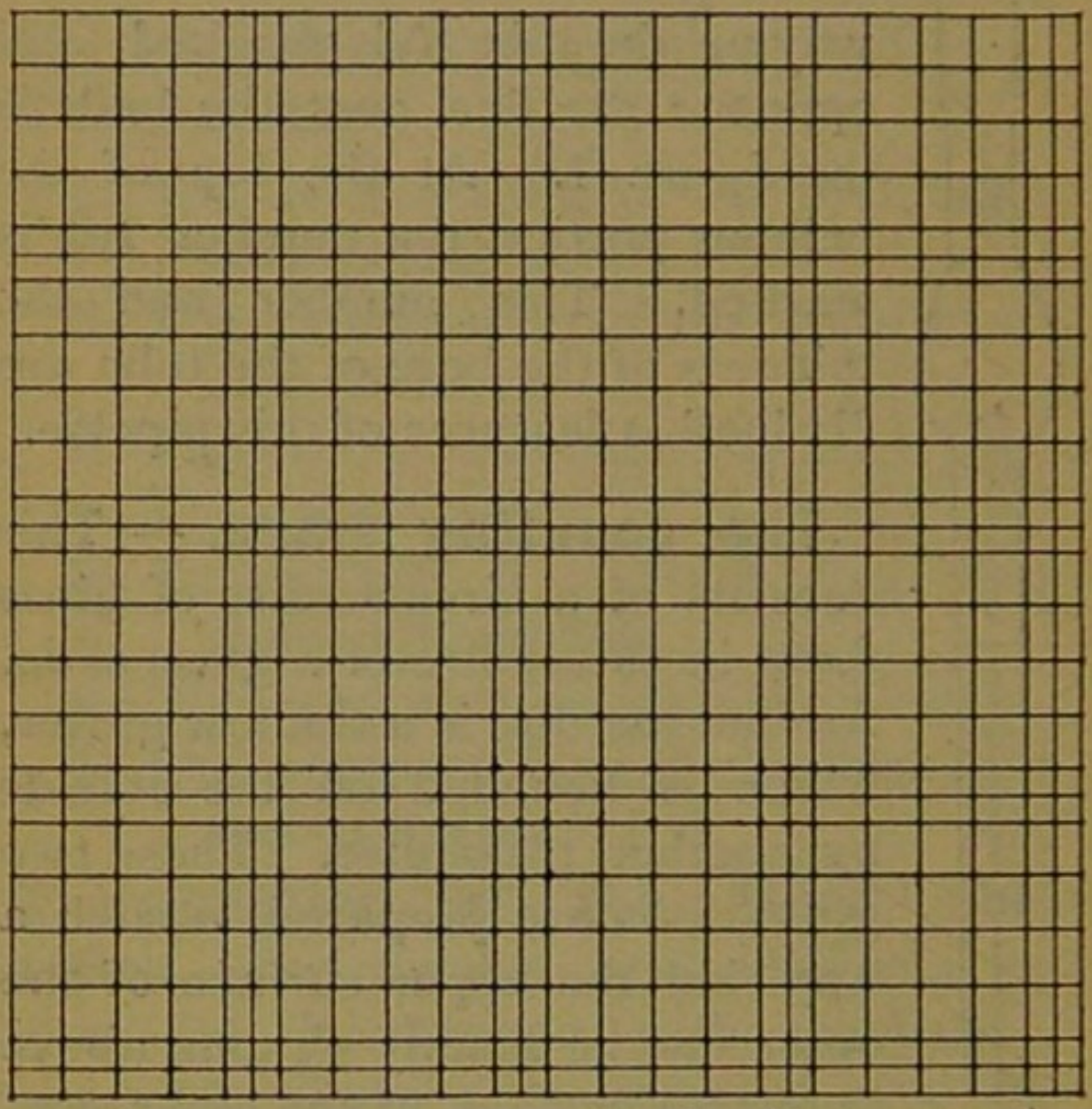


Pipette for counting red blood corpuscles.

a rounded bulbous chamber in which there is a glass bead. The tube is marked off by fine transverse lines dividing it into ten sections. Half-way up the tube ·5 is marked, and opposite the line next the bulb is the figure 1. At the top of the bulbous portion the number 101 is marked. This number and the thinness of the bore of the tube are distinctive features of this pipette.

The counting stage. — This consists of a circular disc of glass fixed in the centre of a glass slide. Around the disc is a shallow gutter, which is bounded on the outside by another glass disc. These two glass discs are prepared in such a way that the upper surface of the outer one is exactly $\frac{1}{10}$ mm. above the upper surface of the inner one. A specially prepared cover-glass is supplied with the instrument. This is laid on to the outer glass disc, and when in position the distance from its under surface to the top of the central disc is exactly $\frac{1}{10}$ mm.

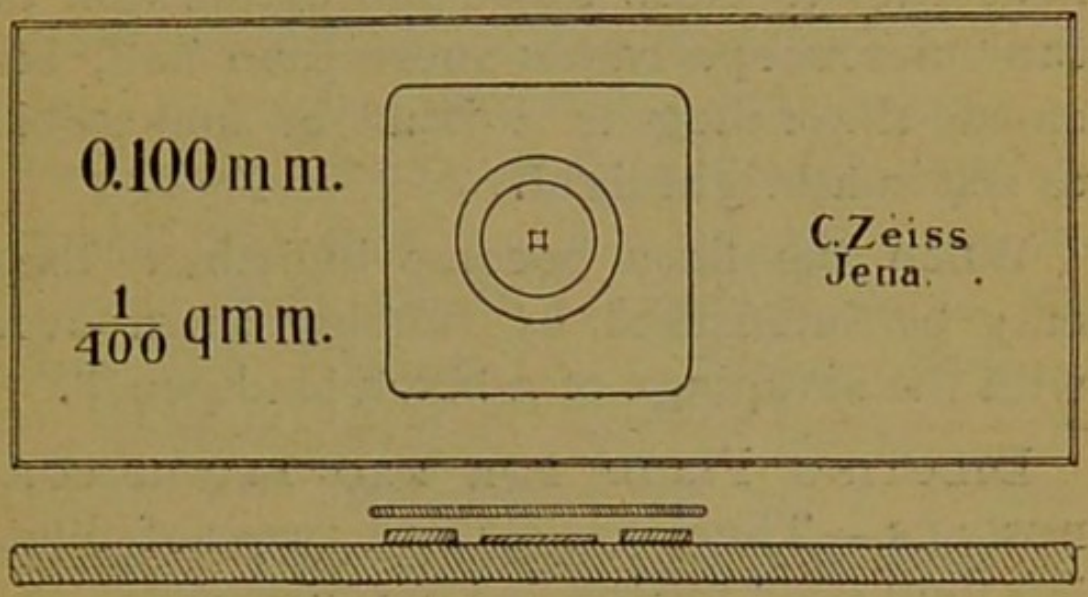
In the Thoma-Zeiss counting stage, the centre of the central glass disc is ruled off into squares. When looked at with a low power of the microscope, it will be seen



1 Square mm. as seen under the "low power." It is divided up into 16 medium squares, each containing 16 small squares.

that the field just includes a large square containing four hundred small squares ruled off in a special manner. This large square is one square millimeter in size. It will be noted that this large square contains

sixteen sets of medium sized squares bounded by lines running in sets of threes. Each of



Counting Chamber to show Central Disc and Cover Glass, etc.

these medium sized squares contains sixteen small squares, each of which measures $\frac{1}{400}$ of a square millimeter. The cubic capacity of a column of fluid standing upon each small square will be $\frac{1}{400} \times \frac{1}{10} = \frac{1}{4000}$ cubic millimetre. The ruling of the stage can be at once appreciated by looking at the diagram.

In using the stage under the microscope there is at times difficulty in finding this ruled portion. This can easily be done by finding the edge of the central disc nearest to the end of the slide—a part always seen at almost any focus—and then running the

slide along till the ruled portion appears.

When examining the counting stage it is necessary to close down the diaphragm of the microscope rather more than half, because the ruling is difficult or impossible to see in a bright light.

When the lines become indistinct, they may be intensified by rubbing them over with the scrapings of a black lead pencil.

DILUTING FLUID FOR RED BLOOD CORPUSCLES.—There are several types of diluting fluid used for the red corpuscles.

Hayem's solution is perhaps the most convenient and generally useful. It has the following formula :—

Perchloride of mercury .	0·5 grm.
Sodium sulphate . . .	5·0 grms.
Sodium chloride . . .	1·0 grm.
Distilled water . . .	200·0 cc.

Enumeration of red blood corpuscles.—Lay out beside the patient :—(1) the pipette, (2) a pricker or Hagedorn needle, (3) bottle of diluting fluid, (4) clean rag, (5) a little methylated spirit or ether.

Cleanse and sterilise the lobe of the ear, or the little finger just above the root of the nail, with the ether or spirit, care being

taken not to produce hyperæmia. Prick the sterile surface and wipe away the first drop of blood. Now take up the red corpuscle pipette and draw up the blood to the mark ·5 on the tube. Wipe away any blood on the outside of the point of the pipette and plunge the nose of the pipette into the diluting fluid which is drawn up to the mark 101. During this diluting process the tube is rotated quickly between the thumb and first finger to allow of free mixing. The corpuscles are now ready for counting.

If it is not convenient to count them at once, an elastic band may be placed over the ends of the tube until the counting is to be carried out.

The tube is thoroughly shaken, care being taken that no fluid is lost in the process. A few drops are now blown out and discarded, and then a drop is placed on the central disc of the counting-stage in such a way that it completely and evenly covers the ruled part, without running over into the gutter around.

The cover glass is then quickly applied taking care to ensure the absence of bubbles. If there be delay in applying the cover

glass too large a proportion of corpuscles would settle on the central ruled portion, due to the surface tension of the drop.

The slide so prepared should be allowed to settle for five minutes before being put under the low power of the microscope. It is a great advantage to have a mechanical stage by which the slide can be centred. The high power (No. 6 Leitz) is now switched on, and it will be seen that the field includes almost exactly one set of sixteen small squares, taking the innermost of the set of three parallel lines in each case as the boundary of the set of sixteen small squares. The corpuscles in this set of sixteen small squares are counted. Corpuscles lying on the boundary lines of two sides are included in the count, while those lying on the other two boundary lines are excluded. With a dilution of 1 in 200 the normal number of corpuscles in the sixteen squares is about 100.

Gulland and Goodall recommend that five sets of sixteen small squares be counted and the results added together. To the total, four noughts (0000) are added, giving the number of red blood corpuscles per cubic millimetre of undiluted blood.

Example—

1st set of 16 small squares				=	92
2nd	,,	,,	,,	=	102
3rd	,,	,,	,,	=	88
4th	,,	,,	,,	=	90
5th	,,	,,	,,	=	100
					472

To 472 are added 0000.

Thus 4,720,000 = R.B.C. per cmm. of undiluted blood.

The simplicity of this calculation depends on the fact that we determine the number of corpuscles in one small square of diluted blood. This means in $\frac{1}{4000}$th of a cmm. We calculate the number of corpuscles in 1cmm. of undiluted blood by the following equation:

$$\frac{x \times 400 \times 10 \times 200}{80} = 4,472,000$$

x = Number of corpuscles in 80 small squares.

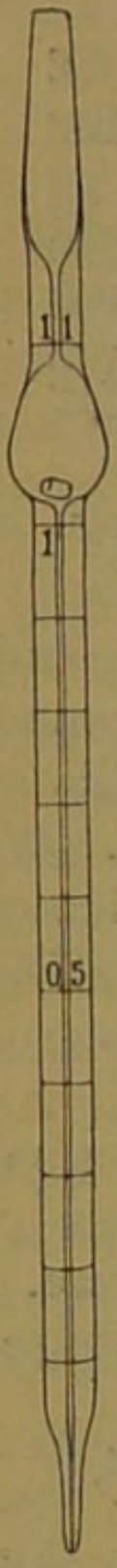
$\frac{1}{400}$ sq. mm. = Area of each small square.

$\frac{1}{10}$ mm. = Depth of drop on counting stage.

1 in 200 = Dilution of blood.

80 = Number of squares counted.

Enumeration of White Blood Corpuscles.—The white counting pipette has a wider bore and smaller bulb than the red corpuscle pipette, and is marked off into ten



sections, the figure ·5 being placed half-way up the tube, and the figure 1 placed just below the bulb. At the top of the bulb is placed the figure 11, which gives another distinguishing mark from the red corpuscle pipette. A dilution of 1 in 10 or 1 in 20 may be taken.

The diluting fluid consists of 1 per cent. glacial acetic acid in distilled water well coloured with methyl-green. The acetic acid renders the corpuscles more visible and the stain assists this by tinting them.

The procedure of drawing the blood, filling the tube, and mixing the contents is the same as for the R.B.C. count, except that a large drop of blood is required to fill the tube to the mark ·5 owing to the wider bore of the tube. A dilution of 1 in 20 is obtained. Special care must be taken to prevent fluid running out of this tube owing to its wider bore.

A drop of diluted blood is put on the counting stage in the same way as for the other tube, but greater care has to be taken

to avoid too large a drop, because of the wide bore of the tube.

The actual counting is done as follows :-- The slide is put under the low power (No. 3 Leitz) of the microscope and the large (1mm.) square "centred." This takes up practically the whole field. The corpuscles are now counted, using the outermost line on each side as the boundary line, beginning at the top left-hand corner and working backwards and forwards along the lines till all the corpuscles are counted which, normally with this dilution, would amount to 40. Corpuscles lying on two boundary lines of the square millimetre are included, those on the other two boundary lines are excluded. Any cells, of course, which lie on lines *inside* the square mm. must be counted.

The number obtained is multiplied by 200 if the dilution was 1 in 20, or by 100 if the dilution was 1 in 10.

The reason for this simple calculation is that 1 sqr. mm. of diluted blood has been counted with a depth of $\frac{1}{10}$ mm. To bring it to cmm. of undiluted blood the number of corpuscles (in 1 square mm. $=x$) must be multiplied by 10 and by 20.. Thus $x \times 10 \times 20$ =number of W.B.C. in 1 cmm. of blood.

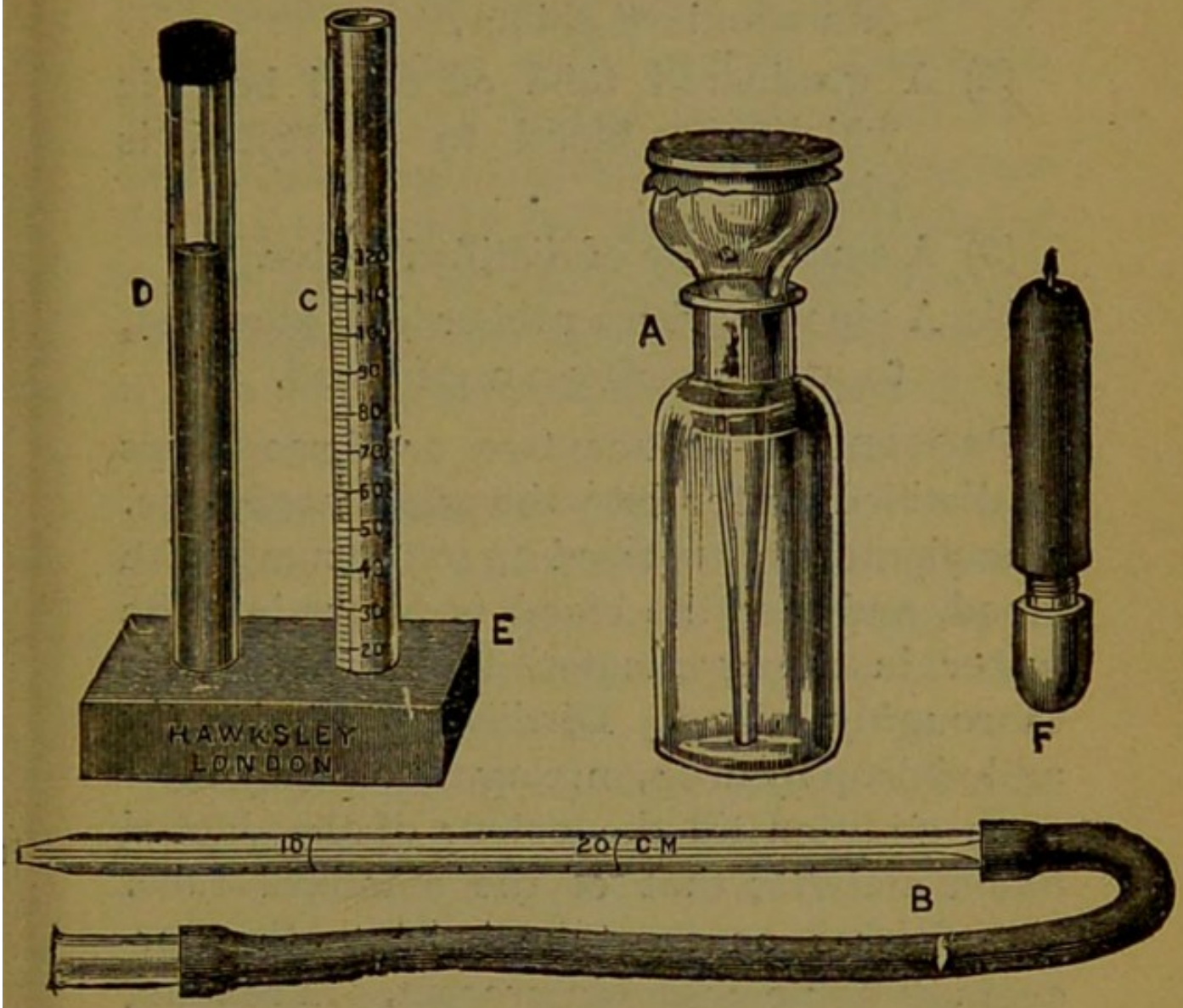
Example.—$40 \times 10 \times 20 = 8000$ W.B.C. per cmm.

Cleaning of Pipettes.—It is of the utmost importance to clean blood-counting pipettes at once after use. If this precaution be neglected clots will form in the tubes which cannot be easily dislodged, and in trying to do so the point of the pipette will almost certainly get chipped, rendering the instrument useless.

Immediately after use, wash out the pipette with distilled water. It is a good plan to wash the tube through the reverse way by placing the rubber tube on the pointed end, and blowing the fluid out at the bulbous end. This washing is followed by absolute alcohol and then by ether in order to render the tube perfectly dry. If a clot forms in the tube which cannot be dislodged by a horse hair, it may be digested by a solution of hydrochloric acid and pepsin.

Estimation of Hæmoglobin.—This can easily be done by means of *Tallqvist's Hæmoglobinometer.* It consists of a book of bibulous papers of a definite uniform consistence, and a coloured scale corresponding to the various percentages of Hæmoglobin.

A paper is moistened with a drop of blood and allowed to just dry (2 mins.). The



GOWER'S HÆMOGLOBINOMETER

paper is then placed behind the scale which is pressed down upon it on the back of the book and compared in good daylight with the scale colours. The tendency of this method is to give rather too high a reading.

Another more accurate method is performed with *Gower's Hæmoglobinometer.*

This instrument consists of:—

(1) A small tube of picrocarmine in gelatin of a standard colour.

(2) A graduated tube of equal bore in which the blood to be tested is placed.

(3) A small bottle of distilled water.

(4) A pipette, with a rubber tube attached, to measure 20 cmm. of blood.

PROCEDURE.—Place two or three drops of distilled water into the graduated tube. The pipette is now filled up to the mark with blood, and then the blood is blown into the water in the graduated tube where it is thoroughly mixed. Distilled water is now added drop by drop, mixing thoroughly after each addition till the colour of the diluted blood matches that of the standard tube. The figures on the tube indicate the percentage of hæmoglobin present. In reading the scale, the tube should be held level with the eyes in daylight against a white background. This instrument is convenient and fairly satisfactory, provided the standard tube is renewed from time to time.

There are several other hæmoglobinometers in use, *Haldane's modification* of Gower's being, perhaps, most frequently

used. The essential difference in Haldane's is that the standard tube consists of 1 per cent. solution of blood with normal Hb. in it and saturated with carbon monoxide. The blood to be tested is converted into carboxyhæmoglobin by simply holding the diluting tube with the blood slightly diluted in such a way that coal gas can pass through a fine tube over the surface of the blood. It is then suitably diluted, and read against the standard.

Colour Index.—The term "colour index" is used to express the proportion of hæmoglobin per corpuscle in a sample of blood. Such an estimation may be of very considerable diagnostic value.

It is obtained by dividing the percentage of hæmoglobin by the percentage number of corpuscles, taking 5,000,000 R.B.C. per cmm. as 100 per cent.

The percentage of corpuscles is obtained by multiplying the first two figures, or first figure in cases under one million, by two. The percentage of hæmoglobin is divided by the figure obtained, thus—

Hb. = 40 per cent.

R.B.C. = 4,800,000 = 96 per cent. $\frac{40}{96}$ = ·4 C.I.

In typical cases of pernicious anæmia the C.I. is above unity.

In typical cases of chlorosis the C.I. is considerably below unity.

Preparation and examination of stained films—Making the blood film.—The blood film should be made on round cover slips of No. 1 type, which must be absolutely clean. The cover slips are cleansed by dropping them singly into nitric acid and leaving them there for half a minute. They are then thoroughly washed in several changes of water to remove all trace of the acid, and finally stored in absolute alcohol till required. Two cover slips are removed from the alcohol and thoroughly dried with a clean soft handkerchief. A small drop of blood is allowed to exude, and the centre of one glass touches and removes the drop without touching the skin. The two cover glasses are now placed in close apposition with the drop of blood between them. They are at once drawn apart by gliding the upper one off the lower one. This must be done without pressing them together too firmly, or lifting them apart.

The films are now dried in the air. For side-room work it is convenient to fix and stain the films at one time, and hence we find a combined fixative and stain, such as Jenner's, most useful.

JENNER'S STAIN consists essentially of a mixture of eosin and methylene blue in pure methyl alcohol (Merk's), the quantity of methylene blue being in excess. The stain may be obtained in tablet form which should be dissolved in methyl alcohol. Jenner's stain should be kept in a bottle with a close-fitting glass stopper. In this way the stain can be kept for months.

The dried blood-film is picked up in Cornet's forceps, and a few drops of Jenner's stain poured on to cover completely the glass slip. The stain is left on for ½ to 1 minute (not longer unless the eosinophil granules are to be specially strongly stained), and then the cover slip is rapidly washed in distilled water till a pinkish colour appears at the edge. During the staining process the glass slip should be covered to prevent evaporation. This may be done by a petri dish.

The glass slip is now set with its edge resting on filter paper to dry at the room temperature. Heat must not be used. The film when dry can be mounted on a slide in Canada balsam.

LEISHMAN'S STAIN may also be used in place of Jenner's. It consists of a "compound of alkaline methylene blue and eosine.

It is dissolved in pure methyl alcohol in ·15 per cent. solution."

The procedure for staining differs slightly from Jenner's method, viz., a few drops of the stain are applied undiluted for ½ to 1 min., and then 10-20 drops of water are added till a pinkish tinge is obtained. This diluted stain is allowed to act for 3 minutes or longer. The film is then washed in ordinary water, dried, and mounted in the usual way.

A Jenner-stained film shows up the various elements in the following colours :—

R.B.C. . . .	greyish terra-cotta.
Nuclei of cells .	blue.
Neutrophil granules	purple.
Eosinophil ,, .	pink.
Basophil ,, .	dark blue.
Blood plates . .	light blue.

Various other methods may be employed in the making and staining of blood films, the above being an outline of the method advised by Gulland and Goodall.

The examination of a stained blood film gives valuable information about all the elements of the blood.

In many diseases it is necessary to make a **"Differential Leucocyte Count."** This gives the percentage of the various types

of leucocytes to one another in a given blood.

It can best be done with the aid of a mechanical stage, because in this way every part of the film can be brought systematically under review. In each field observed, the number of leucocytes present is noted and classified, *e.g.*, polymorphs, eosinophils, lymphocytes, etc. It is well to count at least 500 cells to get a reliable result, and in every important or obscure blood disease, 1000 cells should be counted.

An easy method of doing what at first sight seems a tedious process is to plot out on the left hand side of a sheet of paper letters indicating the type of leucocytes, and then in each field observed place the number of each type of leucocyte present opposite the corresponding letter. This process is continued until 500 are counted, and then the total is divided by 5 to bring the result to a percentage.

Example :—

P. . .	355 = 71	per cent.
L.L. . .	50 = 10	,,
L.S. . .	80 = 16	,,
E. . .	10 = 2	,,
B. . .	5 = 1	,,

Leucocytes.—The average number of

leucocytes per cmm. of blood is about 7000 in an adult male.

The various types of leucocytes which may be present are—

1. Polymorphonuclear Leucocytes.—They are characterised by having an irregularly lobed nucleus which may take on any shape, but is most frequently semi-circular, the lobes being joined by strands of chromatin. The protoplasm consists of a fine reticulum, and contains fine granules staining with acid dyes in most cases, and contrasting with the basic stain for the nucleus. These cells vary much in shape and size. Normally they constitute 65 to 70 per cent. of the total leucocytes.

2. Lymphocytes.—These cells are subdivided into groups known as small lymphocytes and large lymphocytes.

(*a*) *Small lymphocytes* have a rounded nucleus which occupies most of the cell. It stains deeply with basic dyes. The protoplasm may be difficult to make out as it is frequently only a thin circle around the nucleus. The protoplasm stains with basic dyes. The size of an average small lymphocyte is rather less than a red blood corpuscle; they comprise 15 per cent. to 25 per cent. of the total leucocytes.

(*b*) *Large lymphocytes* are much larger than the preceding type of cell. They have a large blue staining nucleus, and have more protoplasm than the small leucocyte. Many of these cells are difficult to classify, for they are essentially transitional types. Normally they constitute about 5 per cent. of the total leucocytes.

3. Eosinophil Leucocytes.—In this type of cell the nucleus usually resembles that of the polymorph, but the protoplasm is full of coarse granules staining red with acid dyes. Normally they constitute from 1 to 5 per cent. of the leucocytes.

4. Basophil Leucocytes. — Mast cells. These cells resemble the eosinophils in their general arrangement, but the protoplasm is packed with granules which stain a deep blue colour with basic dyes. Normally they are present in the proportion of from $\frac{1}{2}$ to 1 per cent.

Myelocytes.—Certain other types of white blood corpuscles are found in pathological states of the blood:—

1. Neutrophil Myelocytes occur normally in bone marrow, and ultimately form the normal polymorphs of the blood. They may occur pathologically in the blood, and in stained specimens show a somewhat oval,

slightly indented nucleus. The protoplasm contains fine neutrophil granules.

2. EOSINOPHIL MYELOCYTES are the same as the above, except that the granules in the protoplasm stain with acid stains.

3. BASOPHIL MYELOCYTES show marked basophilic staining in the protoplasm.

Red Blood Corpuscles—Abnormalities—These abnormalities may arise in certain diseases. The following is a list of the commoner of abnormal red cells :—

1. MEGALOBLAST
   NORMOBLAST
   MICROBLAST
   } These are nucleated red cells which may be larger, the same size, or smaller than an ordinary red corpuscle, and have a normal outline.

2. MACROCYTE
   MICROCYTE
   } These terms are applied to red cells depending on whether they are larger or smaller than a normal red corpuscle—a normocyte.

POIKILOCYTE is a term applied to a red blood corpuscle when its shape is irregular.

Polychromatic staining signifies that the cells react to both the acid and basic stains giving rise to a kind of greyish purple colour.

Punctate basophilia is a term applied to a red blood corpuscle in which many basophilic dots can be seen throughout the

protoplasm. It is probably a degenerative phenomenon occurring in toxic states of the blood.

The glycogen reaction.—Gulland has drawn attention to the value of this reaction in diagnosis. It has been shown that in certain diseased conditions glycogen can be demonstrated in the protoplasm of polymorphonuclear leucocytes.

The following solution is required to bring out the reaction :—

Iodine	grm.	i.
Potassium iodide . .	grms.	iii.
Distilled water . .	cc.	100.

Gum arabic is added to make the fluid syrupy.

A blood film is made in the usual way on an ordinary microscope slide. It is allowed to dry and then a drop of reagent is placed on the slide. After about one minute a cover slip is placed on the drop and pressed down on the slide. The preparation is now ready to be examined with an oil-immersion lens by means of daylight.

Normal leucocytes stained in this method have a faint yellowish appearance, the nuclei staining more darkly than the protoplasm.

THE REACTION shows itself in the protoplasm of the polymorphs only, and may be one of three degrees, viz. :—

(1.) Mild reaction—a diffuse brownish colouration in the protoplasm.

(2.) Medium reaction — fine brownish granules which may be throughout the cell.

(3.) Marked reaction — coarse brown granules throughout the cell.

SIGNIFICANCE OF THE REACTION.—The reaction is indicative of some serious bodily disturbance, and is met with in cases of suppuration and bacterial infection such as pneumonia, empyema, appendicitis, peritonitis, etc.

Coagulation-time of the blood.—The estimation of how long blood takes to coagulate may be done in one of many ways. Coagulation-time does not mean the time taken for blood to clot in a wound, but the time taken for fibrin to form in blood drawn from the body into an instrument.

WRIGHT'S METHOD is simple and easily carried out. A series of tubes with diameter of ·25 mm. is taken and placed in water at body temperature. The tubes are then taken out at intervals of one minute, and a

column of blood drawn up into each tube. Each tube is numbered and replaced in the water tank. The tubes are now taken out at stated intervals and the contents blown out on blotting paper to note the earliest sign of clotting. This process goes on until the tube is reached where the blood cannot be expelled. The length of time between the filling of the tube and the time when the blood cannot be blown out is taken as the coagulation-time.

We shall here summarise the articles required to be taken to a patient's house in order to perform a thorough blood examination :—

1. The hæmoglobinometer case with its pipette, distilled water and tubes or Tallqvist's book.
2. The case with the "red" and "white" counting pipettes. This case should contain a pricker, flat elastic bands, and an old linen rag. These can be put in the space for the counting chamber, which is left at home.
3. Diluting fluids for red and white corpuscles in small half-ounce bottles which must be perfectly clean,

4. A few microscope slides and clean cover glasses.
5. Occasionally the glycogen reagent may be required.
6. Capillary pipettes in some cases.

Specific Gravity of Blood.—A convenient and fairly accurate method of estimating the specific gravity of blood has been introduced by Hammerschlag.

He uses a mixture of equal quantities of benzol (specific gravity 888) and chloroform (specific gravity 1480) in a glass cylinder. A drop of blood is dropped into this mixture, and if it floats benzol is added, if it sinks chloroform is added. One or other solution is added until the drop is just suspended in the fluid. After each addition of benzol or chloroform the mixture must be well stirred.

The specific gravity of the mixture is taken with a specially delicate instrument when the mixture holds the drop in correct suspension.

Hammerschlag's procedure has to be done quickly, as evaporation and changes of temperature would give erroneous results.

The normal specific gravity of blood is 1058.

WIDAL'S REACTION

This reaction is employed in the diagnosis of typhoid fever and is frequently known as an agglutination test, because the principle of its reaction depends on the fact that the serum of a patient suffering from typhoid fever develops certain substances known as agglutinins. These substances have the power of inhibiting the motility of the B. typhosus organism.

The reaction should be carried out in a laboratory. Only an outline of the procedure of conducting the test will be given here.

REQUISITES:—1. An 18 hour culture of typhoid bacilli; 2. Pipettes; 3. Watch glass; 4. Slide and cover glass for a hanging drop preparation; 5. Normal saline.

PROCEDURE IN BRIEF—A puncture is made in the ear and blood allowed to flow into a small pipette, the end of which has been drawn out as a capillary tube.

The ends of the pipette are sealed off in the flame.

An emulsion of the typhoid bacilli is made and diluted 1:10 with normal saline. This may be done by mixing one platinum needle loopful of typhoid emulsion with nine loop-

fuls of saline. A drop of serum is taken in the same way from the pipette and diluted 1:10.

A loopful of dilute emulsion and dilute serum are now mixed on a cover slip and examined as a hanging drop preparation. This is examined under a high power of the microscope, and the organisms are usually seen to run into clumps in from 5 mins. to 2 hours' time.

Various weaker dilutions should also be made up. A reaction is considered positive when clumping takes place in the same time with a dilution of 1·50.

THE SPUTUM

The examination of the sputum includes:—

1. The inspection of the expectoration.
2. The microscopic characteristics, including bacteria present.

Inspection and significance of certain definite characteristics of the sputum:—While inspecting fresh sputum it is well to keep in mind that constituents from the œsophagus, nose, mouth, etc., may be lodged in the expectoration coming from some part of the respiratory tract.

THE AMOUNT OF SPUTUM.—It is necessary in most cases of pulmonary disturbance to note the quantity of sputum brought up and to note if there is more at any special ime of the day or night.

Scanty sputum is noted in early stages of bronchitis, pneumonia, pulmonary tuberculosis, and some cases of asthma.

Profuse sputum is characteristic of chronic bronchitis, resolving pneumonias, advanced pulmonary tuberculosis, bronchiectasis, gangrene of lung, and pulmonary abscess.

MUCOID SPUTUM owes its glairy, tenacious, and clear appearance to the large amount

of mucin present. Such sputum forms a cloud on the addition of acetic acid. It is typical of acute bronchitis, whooping cough, or certain cases of asthma.

MUCOPURULENT SPUTUM, as its name suggests, is produced by a combination of mucus and pus in varying proportions. At times the pus appears as small pin heads in the mucus, at other times the two constituents are freely mixed. It is characteristic of pulmonary tuberculosis, resolving pneumonia, any suppurative condition in the lung, etc.

PURULENT SPUTUM is composed almost entirely of pus and is characteristic of an abscess which has ruptured into the lung, or developed in the lung from the first.

SEROUS SPUTUM contains much albumin, is clear and frothy. It is met with in œdema of the lung or a perforated pleurisy.

NUMMULAR SPUTUM is characterised by having tough, coin-like masses floating in it. These masses are muco-purulent and occur in patients who suffer from tuberculous cavities.

BLOOD IN SPUTUM may occur in large or small quantities. The blood may cause only a streaking of the sputum, or may gush up in mouthfuls. It is found in many different

lung diseases, *e.g.*, tuberculosis, pneumonia, congestion. It is met with in trauma, *e.g.*, fractured rib, wounds, etc. It is met with also in circulatory diseases, *e.g.*, aneurysm, mitral stenosis, etc.

Rusty sputum is a term applied to the expectoration of a pneumonic patient, and is caused by the passage of blood from the congested alveolar capillaries.

Prune-juice coloured sputum may occur in severe cases of pneumonia and in gangrene of the lung.

The sputum may be coloured in various ways by derivatives of hæmoglobin. In cases of jaundice it may appear in various shades of green.

When a large quantity of sputum is being voided, if it be placed in a conical glass, it frequently divides up into three distinct layers. The lowest layer consists largely of detritus of lung tissue, the middle layer of watery fluid, and the top layer of frothy mucus. When sputum separates out in this way, it indicates putrid bronchitis or gangrene of the lung, etc.

The Odour.—In most pathological conditions of the lungs, except, perhaps, in a pure case of bronchitis, the expectoration has some smell varying from a mildly per-

ceptible odour to an excessively foul smell in a sputum from a rapidly breaking down lung.

Macroscopic elements in sputum other than pus and mucous plugs.—(1) NECROTIC PORTIONS OF LUNG tissue may be demonstrated by squeezing a portion of the sputum between two glass slides and observing fine yellowish filaments of elastic tissue.

(2) DITTRICH'S PLUGS.—They are casts of the bronchi, varying considerably in shape, and dependent upon the size of the bronchus in which they were formed. When broken between the fingers they are horribly foul smelling. With the aid of the microscope it has been shown that these plugs consist of fatty acid crystals, bacteria, fat droplets, and cell detritus.

(3) CURSHMANN'S SPIRALS are casts of the fine bronchi formed of spirally wound mucin enclosing cells of various types, and in some cases crystals. When seen in sputum they occur as little balls like sago-grains. These can be teased out into a long shred of mucin. These spirals are seen typically in the early stages of many cases of bronchial asthma, and are supposed to be due to a fine type

of bronchiolitis. They have also been seen in other lung diseases.

(4) FIBRINOUS CASTS form in the bronchi in some cases of pneumonia, bronchitis, etc. They are coughed up in a rolled-up form, but when floated out in water assume the shape of the bronchus and its branches from which they were coughed.

Microscopical examination of the sputum.—This gives valuable information as to the presence of foreign matters, moulds, elastic tissue, debris, pus, etc.

CHARCOT-LEYDEN CRYSTALS occur in the sputum of asthmatics. They are composed of organic phosphate, are colourless, sharply pointed, and octahedral in shape. Fatty acid crystals are also found in sputum from cases of gangrene, putrid bronchitis, and bronchiectasis.

The bacteria can also be determined by staining a film in a suitable manner, and the various types of organisms can be determined by "plate culture."

A short description will now be given of the method employed in staining sputum for the presence of certain of the commoner organisms pathologically present.

Tubercle Bacilli.—The method here

described is that employed by Dr Miller at the Royal Victoria Dispensary, Edinburgh.

The patient expectorates the sputum first brought up in the morning into a small wide-necked bottle which has been rendered perfectly clean. The sputum is now transferred to a clean Petri dish, and a thickish nummular portion of sputum selected. By means of a pair of forceps and scissors, a portion is transferred to a *large* microscope slide. Another slide is placed on the top, and the two squeezed together and drawn apart. Both slides are now dried well above the flame, and then fixed by passing six times through the flame.

The films are now stained by the Ziehl-Neelsen method :—

(1) The following solution is made up and filtered on to the films.

Basic fuchsin . . .	1 part.
Absolute alcohol . . .	10 parts.
Carbolic acid in water (1·20)	100 parts.

(2) Heat gently till steam begins to rise and keep just steaming for 3-5 minutes.

(3) Decolorise the films in acid alcohol (*i.e.*, 1 per cent. conc. HCl. in methylated spirit) till a faint pink

is seen at the thinner portions of the film. This takes ½–1 mins.

(4) Wash the slides thoroughly in water.

(5) Counter-stain for half-a-minute in 1 per cent. solution of methylene blue.

(6) Rinse in water.

(7) Dry the film thoroughly by wiping away the bulk of the water and then drying high over the flame.

(8) The film is now ready to be examined with the oil-immersion lens. If a permanent preparation is required, the film may be mounted in xylol-balsam in the usual way.

Each slide should be carefully searched for tubercle bacilli. At least five minutes by the clock should be taken to each slide. Tubercle bacilli show as short rods staining a red colour. If a suspected sputum is negative after being subjected to the above examination, a further test can be carried out.

This is done by means of antiformin. Antiformin is made by mixing equal quantities of the two following solutions :—

(1)	Sodium carbonate .	10 grms.
	Chlorinated lime .	50 ,,
	Distilled water .	100 cc.
(2)	Sodium hydrate .	15 grms.
	Distilled water .	100 cc.

The most recent and probably the most valuable way of using this antiformin method is described by Eürich.

Sputum is mixed with antiformin in about equal proportions, if the sputum be very tenacious. If less tenacious then less antiformin is required to dissolve it. The mixture is now diluted with ten times its bulk of water and placed in a tall cylinder. Acetone and ether (methyl ether will do) are now mixed in equal quantities, and the same quantity of this mixture as that of the water is added to the glass cylinder.

The whole is now thoroughly shaken and allowed to settle, when it will be seen that a white ring separates out at the junction of the fluids. This contains any tubercle bacilli present along with any other undissolved matters in the sputum. The white deposit is pipetted off and films of it made in the usual way, or it may be centrifuged and films made from the deposit.

This method has the advantage over some others that the process can be quickly done,

that it can be stopped at any stage, and that it is a cleanly way. Tubercle bacilli have been found in a white ring which was left for 6-7 days before being pipetted off for film examination.

Diplococcus Pneumoniæ.—This organism can be demonstrated in the mouth of many normal individuals, as well as those suffering from lobar pneumonia.

It can be demonstrated in a film preparation of sputum in several ways.

1. GRAM'S METHOD.—The film of sputum is dried and fixed above, the flame. The film is then stained in a 1 : 10 alcoholic solution of carbol gentian violet for one minute. Gram's iodine is now added and allowed to remain on for one minute. It is differentiated in methylated spirit for a few seconds and then washed in water. A counter stain such as ½ per cent. watery solution of safranin is added. After half a minute's staining the film is well washed in water, then dried high above the flame and mounted in xylol balsam.

Pneumococci and all other Gram-positive bacteria are stained purple, other bacteria, etc., are stained red.

2. CARBOL-THIONIN BLUE.—This is a standard method of showing up the large proportion of the various bacteria.

(1) A film is made, dried, and fixed in the usual way.

(2) Thionin blue solution—1 grm. thionin blue in 100cc. carbolic acid (1 in 40)—is dropped on and allowed to stain for 3–5 minutes.

(3) Wash well in water.

(4) Dry and mount in the usual way.

Bacteria are stained a deep blue colour. In the case of capsulated organisms a clear or faint blue halo is seen around them.

3. Hiss's Capsule Staining Method.—

(1) Films are made and fixed in the usual way.

(2) One part of a saturated alcoholic solution of fuchsin is added to nineteen parts of distilled water. This stain is dropped on to the film and heated for a few seconds till steam rises.

(3) The stain is washed off with a 20 per cent. copper sulphate solution.

(4) The slide is thoroughly dried between filter papers without any previous washing.

The centre of the organism is stained a red colour, and the capsule a delicate blue. There are various other methods of staining capsules, but the above has proved easy and satisfactory.

Friedlander's pneumobacillus.—This organism is the causative factor in some cases of pneumonia.

It resembles the pneumococcus in occurring in pairs, and in having a capsule. It differs in being Gram-negative, and is a more elongated organism varying in size. It stains with the ordinary anilin dyes.

Streptococci are frequently found in sputum. They occur as rounded organisms in short and long chains. They stain with the ordinary dyes and are Gram-positive. They usually have no capsule.

Staphylococci occur in bunches of organisms. They stain with the ordinary dyes, are Gram-positive and have no capsule. They may set up bronchitis in a certain number of cases.

Diphtheria bacilli are to be found in the sputum of diphtheritic patients when membrane is coughed up. They occur in the membrane which develops on the throat.

A direct film may be made from the throat swab by the inoculation of the culture media, but this organism is best demonstrated by means of NEISSER'S STAINING METHOD after cultivation. This requires two reagents :—

1.	Methylene blue . . .	1 grm.
	Alcohol 96 per cent. . .	20 cc.
	Glacial acetic acid . .	50 ,,
	Distilled water . .	950 ,,

2. Bismarck brown . . 2 grms.
   Distilled water . . . 1000 cc.

(1) Stain the film made from the culture tube for 1–3 seconds in the methylene blue solution.
(2) Wash quickly in water.
(3) Stain for 3–5 seconds in Bismarck brown.
(4) Wash quickly in water, dry and mount.

The typical appearance of the bacilli is brownish with one to three blue granules present in each.

Influenza bacilli are found in the sputum of patients suffering from the early stages of influenza.

The bacillus is a very minute organism found free in the sputum or within pus or epithelial cells. It is Gram-negative and stains well with carbol-fuchsin. It cannot be identified with certainty till cultures are made.

If a full bacteriological examination of the sputum is required, culture media must be inoculated, and when the different colonies are grown their morphology can be studied. The details of this process cannot be dealt with here.

PUS

The collection of pus for examination during an operation is easy. The pus is allowed to run into a sterile test tube, and from this cultures and films can be made.

When pus has to be withdrawn from an abscess for examination by culture, etc., it is convenient to use a Wright's pipette with a nipple at the upper end to withdraw the fluid. The abscess is opened and the pipette is inserted so that the sides of the opening in the skin are not touched. The tube can now be sealed up to be sent to a laboratory, or films and cultures can be made at once.

PREPARATION OF FILMS.—A drop of pus is put on a slide and spread well with a sterile platinum needle, dried, and fixed in the same way as a film of sputum. It can now be stained by (1) carbol-thionin blue or methylene blue in watery solution; (2) by Gram's method. The latter method is of great value for diagnostic purposes.

Agar, gelatin, and broth culture tubes should also be inoculated, and if thought advisable, a drop of the pus should be "plated out."

These are strictly laboratory methods, and as yet do not come under the designation of "side-room" work.

Many organisms give rise to pus. Amongst the more important are staphococci, streptococci, pneumococci, and gonococci, B. typhosus, B. mallei, B. coli communis, B. pyocyaneus, and the fungus of actinomyces.

GASTRIC CONTENTS

In many diseased conditions of the alimentary tract, a complete examination of the "stomach contents" gives valuable information from a diagnostic and therapeutic point of view.

Such an examination includes :—

1. Physical examination of a test meal.
2. Chemical ,, ,,
3. Microscopical ,, ,,

1. **Physical Examination.**—In order to obtain as reliable results as possible it is necessary to examine the contents of the stomach after a certain definite quantity and type of food have been taken. This also enables one to estimate comparative results.

Various workers in this domain of medicine have introduced what are termed "*test meals*" by which a definite standard of food is given to the patient, and allowed to remain a certain time in the stomach before being withdrawn for examination.

These test meals are usually given first thing in the morning, *i.e.*, on an empty stomach, and are made to simulate to a certain extent an ordinary simple meal

typical of the country to which the author belongs who has suggested it.

Examples of Test Meals.—(1) EWALD'S TEST BREAKFAST consists of a roll of bread or thick slice of toast (2 ℥), and a breakfast-cupful of weak tea without sugar or cream. The contents are drawn off at the end of an hour.

2. FISCHER'S TEST BREAKFAST consists of the bread and tea (or water) of the Ewald meal plus ¼ lb. finely minced lean meat broiled or slightly seasoned. It is removed in three hours.

3. RIEGEL'S TEST MEAL.—A plate of meat soup.

Beef steak .	5–7 ozs. (150–200 grms.)
Potato puree .	5¼ ozs. (150 grms.)

It is removed in from three to four hours. In seven hours the meal should have completely left the stomach.

4. BOAS'S TEST SUPPER consists of 2 oz. bread and butter with slices of cold meat and a cupful of tea with sugar if desired. This meal is given at night, and the stomach washed out next morning. The sediment is examined by the microscope for undigested food.

It will be seen from the above examples

of test meals that there is considerable variety of type, but most workers on gastric analysis, be they British, German, or American, insist on the importance of several minor points in the giving of test meals, viz. :—

1. The test meal should be as nearly as possible a meal to which the patient is accustomed.

2. It should be given at a time of the day when the patient is accustomed to take a meal of that type.

3. The type of patient we have to deal with must be remembered when we are giving a test meal.

4. Many authors lay stress on having the stomach thoroughly empty before a test meal is given—this point is disputed by others. A compromise is probably the best way, by taking for granted that after a light supper the stomach will be practically empty by morning, and so ready for the test breakfast.

5. Never trust much to the analysis of the first test meal which a patient receives, as subjective sensations are apt considerably to upset acid secretion, especially in nervous people.

6. Do not hold too strictly to the time

a meal may remain in the stomach before being withdrawn for testing purposes. The meal should be withdrawn at the time of maximum secretion.

From the above it will be seen that in the past we have paid too little attention to the effect of the mind on gastric secretion, and while the Ewald breakfast may give valuable aid as to the quantity of acid secreted, one should look on this as a minimum quantity in a British or American stomach, which is accustomed to a more substantial breakfast.

THE REMOVAL OF THE "STOMACH CONTENTS"

This is done by means of an ordinary stomach tube of sufficient length to allow the stomach contents to be removed into some vessel.

The tube should be lubricated with milk or glycerine and pushed well back into the pharynx. The patient is told to swallow, and as he does so, the tube is gradually pushed down the gullet. The patient at first usually suffers from very nauseating symptoms, which, however, may be diminished in severity by his taking long breaths. The contents may well up at once, or may be

expelled in efforts to vomit. It may be necessary to aspirate off the contents.

The stomach contents are now placed in a Y-shaped glass and inspected to note the quantity returned, the amount of semi-digested solid, the quantity of fluid, the presence of any abnormal constituent in bulk, *e.g.*, pus, blood, mucous, etc.

It is of value to note that the quantity withdrawn in a certain time may give information as to the digestive power, for the quicker the digestion the less fluid will be withdrawn.

No details can here be given as to the physical characters of the various contents withdrawn. Boas estimates that from 20cc.-50cc. may be regarded as a normal quantity to be withdrawn after a German test breakfast. If the quantity withdrawn is over 150cc. hypomotility must be present.

CHEMICAL EXAMINATION

The chemical examination is now undertaken, and for this purpose the gastric contents must be filtered through muslin, the filtrate being used for the test.

Normal gastric juice has the following characters :—

Gastric juice is a clear fluid, with a specific

gravity between 1002 and 1009, in which the important chemical constituents are hydrochloric acid and pepsin. The proportion of hydrochloric acid is given by various authors between limits of ·18 per cent. and ·42 per cent. When digestion is in progress, this proportion is greatly lowered by combination of the free hydrochloric acid with protein bodies to form acid albumins.

The stomach contents are mixed with acids taken with the food, with mucus, with saliva, and, perhaps, with regurgitated bile and pancreatic juice. Part of the acidity is also due to the presence of acid salts, especially acid phosphate of soda and the organic acid which may be formed by decomposition processes.

The normal total acidity of stomach contents after a test meal is from 40 to 60, as estimated by the number of cubic centimetres of decinormal soda solution required to neutralise 100 cc. of stomach contents, corresponding to a hydrochloric acid content of between ·1 per cent. and ·2 per cent.

The following questions as to the reaction of the "contents" must be determined :—

1. Are the "contents" acid?

2. How acid are the "contents"? (total acidity.)
3. Is free HCl present?
4. How much free HCl?
5. How much combined HCl?
6. How much acid salts?
7. Are organic acids present?

1. Are the "contents" acid? Test them with litmus paper. They are usually acid. In certain circumstances they may be alkaline or neutral.

2. How acid are the "contents"? (total acidity)

PROCEDURE.—10cc. of the filtered gastric contents are placed in a porcelain dish and diluted with from 30 to 40cc. distilled water. Two or three drops of a 1 per cent. alcoholic solution of phenol-phthalein are added to the diluted gastric contents. Above the porcelain dish a burette is fitted and filled with $\frac{N}{10}$ NaOH. The height of the $\frac{N}{10}$ NaOH solution is noted, and then the stopcock of the burette is turned to allow the alkali to run into the gastric contents, stirring all the time, until a permanent pinkish-red colour develops. The burette is again read off and the height of the fluid noted. The difference between the readings gives the num-

ber of cc. $\frac{N}{10}$ NaOH required to neutralise 10cc. filtered gastric contents.

The number of cc. required to neutralise 100cc. of stomach contents gives us the total acidity in Ewald's notation.

EXAMPLE :—3·4cc. $\frac{N}{10}$ NaOH have been used to neutralise 10cc. of stomach contents. Thus 34cc. would be required to neutralise 100cc. gastric contents, *i.e.*, the total acidity would be 34 in Ewald's notation.

It has been suggested that the term "acidity per cent." be applied to a figure obtained in this way, which indicates the amount of alkali used to neutralise 100cc. of acid contents.

Another method of expressing the result is in terms of HCl equivalent to the alkali used. In this case the number of cc. of $\frac{N}{10}$ NaOH used are multiplied by ·00365 grm. to equal the amount of HCl by weight in 10cc. This will give percentage in terms of HCl, and in the above example would read ·0125 in 10cc., or ·125 per cent. of HCl. It must be kept in mind that the acidity is not entirely due to HCl.

3. Is free hydrochloric acid present? This may be determined in one of several ways by using a reagent which changes

colour when brought into contact with free acid.

(1) CONGO-RED PAPER.—A strip of this paper is dipped in the filtered "contents," and if hydrochloric acid be present the red colour will change to a deep blue. This blue paper is now dipped in ether and the colour will be seen to remain.

If organic acids are present in large amount, they will turn the congo paper a grey blue colour which disappears on dipping the paper into ether and dissolving up the organic acid. This test is convenient and satisfactory.

(2) TROPÆOLIN 00 PAPER is used like congo-red paper, or the reagent may be kept in a watery solution. The natural straw colour of the reagent is turned to brown in the presence of free mineral acids. The more acid there is the deeper will be the brown colour.

(3) DIMETHYL-AMIDOAZO-BENZOL (TÖPFER'S REAGENT) in a 5 per cent. alcoholic solution is of a pale brown colour which becomes bright red on contact with hydrochloric acid. A few drops of the stomach contents are placed in a porcelain dish and diluted with distilled water. To this is added a drop of the reagent which will

turn red in the presence of free acid.

(4) PHLOROGLUCIN AND VANILLIN (GÜNZBERG'S REAGENT) is made up thus :—

Phloroglucin . . .	6 parts.
Vanillin	3 ,,
Absolute alcohol . .	90 ,,

A few drops of this reagent are placed in a porcelain dish and very carefully evaporated. A drop of stomach contents is brought in contact with the reagent and warming continued. If free mineral acid be present a rose-red colour develops when evaporation is complete. Care must be taken not to overheat and char the solution.

(5) BOAS'S REAGENT consists of :—

Resorcin	5 grms.
Cane sugar . . .	3 ,,
50 per cent. alcohol . .	100 cc.

It is used in the same way as Günzberg's reagent giving a red colour in the presence of free HCl. It is less expensive and keeps well.

(4) **How much free hydrochloric acid is present?** This may be simply determined by using dimethyl-amidoazobenzol as an indicator. A burette is filled with $\frac{N}{10}$ NaOH, 10cc. of filtered stomach

contents are placed in a porcelain dish and diluted with distilled water. To this is added a minute drop of Töpfer's reagent which gives a clear red colour to the "contents." The alkali is now run in slowly until the red colour changes to a canary yellow. It requires some practice to determine the exact point when the change takes place, the important point being to note exactly when the last trace of red has gone. The estimation may be read as in the total acidity estimation as percentage of HCl or "acidity per cent." as in Ewald's notation. The amount of alkali used is noted, and the percentage of free HCl determined by multiplying the figure obtained by ·0365.

If a drop or two of phenol-phthalein solution be now added to the neutralised stomach contents, the total acidity may be determined by further addition of the alkali till a pink colour is produced indicating complete neutralisation of all acidity in the specimen.

This can be more easily understood by reference to the following example :—

The decinormal soda solution in the burette stands at 21. 10cc. of filtered stomach contents are taken (diluted with 30 or 40cc. of distilled water) one small drop of "dimethyl"

solution is added and the $\frac{N}{10}$ NaOH is run in until the red colour is just, but completely, lost. The reading on the burette is again taken and found to be 26·5. Two to three drops of phenol-phthalein are added, and more decinormal solution run in until a rose red tint appears and remains distinct after stirring.

The burette reading is again taken and found to be 27.

The amount used for the dimethyl titration is 26·5 – 21 = 5·5cc., and the amount for the phenol-phthalein titration 27 – 21 = 6cc. The total acidity is therefore 60, and the hydrochloric acidity 55.

The conversion into percentage depends upon the following :—

5·5cc. $\frac{N}{10}$ NaOH = 10cc. STOMACH CONTENTS.

55cc. $\frac{N}{10}$ NaOH = 100 ,, ,,

5·5cc. normal NaOH = 100 ,, ,,

$\frac{5{\cdot}5 \times 23 + 16 + 1 \text{ grm.}}{1000}$ NaOH = 100cc. ,,

$\frac{5{\cdot}5 \times 1 + 35{\cdot}5 \text{ grms.}}{1000}$ HCl in 100 ,, ,,

i.e., 5·5 × ·0365 = ·2 per cent. HCl.

Therefore, to find the percentage of hydrochloric acid present, multiply the number of ccs. of decinormal soda solution required to

neutralise 10cc. gastric contents by the constant factor ·0365.

5. **How much combined hydrochloric acid is present?**—PROCEDURE.—This may be estimated by using a special indicator. A measured quantity (10cc.) of stomach contents is placed in a porcelain dish, and to this is added a few drops of a 1 per cent. solution of sodium alizarin sulphonate. Decinormal soda solution is run in until a violet colour appears, due to the neutralisation of all the constituents of the total acidity except the loosely combined acid albumin. The amount of decinormal soda solution used gives the amount of acidity due to all the other factors (free acid and acid salts); by subtracting this from the total acidity already found we obtain the amount of the combined acid. This is a simple method, but not very trustworthy, because the alizarin solution does not keep well, and the change of colour takes place slowly, not giving a very sharp reaction.

6. **How much of the acidity is due to acid salts and organic acid?**—This may be determined by subtracting the sum of the free and combined acidities from the total acidity.

7. **Are Organic Acids present?**—Lac-

tic, butyric, or acetic acid may be found in stomach contents.

Lactic Acid is more frequently found in gastric contents than the other two. It results from fermentation, and occurs in cases of deficient HCl and in gastric stasis. It is important when testing for this acid to be sure that no lactic acid was given with the test meal.

The most commonly used qualitative estimation for lactic acid is *Uffelmann's test.*

The following solution is prepared freshly each time:—

1 per cent. carbolic acid 20cc.
10 per cent. ferric chloride solution 1-3 drops.
An amethyst colour is produced.

The fluid is now diluted till it becomes fairly clear. A test tube is now half filled with the reagent and a few drops of the stomach contents are added. If lactic acid be present the blue colour is discharged and a canary-yellow takes its place. A control may be done in another test tube with a drop of distilled water instead of the gastric contents.

Butyric Acid.—It is seldom necessary to test for butyric acid as its smell (like rancid butter) is sufficiently characteristic. A few grains of calcium chloride added to the

"contents" will cause the separation of oily droplets if butyric acid be present.

Acetic Acid.—This acid may be recognised by its odour.

The following test may be employed:—The acid is carefully neutralised with sodium hydrate, and to this is added a drop or two of ferric chloride which gives a blue reaction due to the formation of ferric acetate.

It will now be obvious that we can determine the following facts by titration in a fairly accurate manner, viz.:—

1. Total acidity by means of phenolphthalein.
2. Total quantity of free HCl by the dimethyl method.
3. Total quantity of free acid and acid salts by the alizarin method.
4. Total quantity of acid albumin by subtracting the quantity of free acid and acid salts from the total acidity.
5. Total quantity of acid salts and organic acids by a subtraction process.

Method of determinining acid deficiency.—In cases where free HCl is absent it may be necessary to determine the extent

of the excess of combinable material over the HCl, if any, already combined. This deficiency in "HCl saturation," as Sahli calls it, can be determined by titrating $\frac{N}{10}$ HCl against a definite volume of stomach contents to which Grünzberg's reagent has been added.

It has been determined that the combining capacity of an ordinary Ewald test breakfast amounts to 20cc. of decinormal hydrochloric acid for every 100cc. of "contents," *i.e.*, an acidity of 20. Therefore, in a stomach secreting absolutely no acid, about 20cc. $\frac{N}{10}$ HCl would require to be added before the reaction for free acid would appear, the acid up to this point combining with the bases and protein bodies. The number of ccs. required to bring out this reaction subtracted from 20 would give an idea of the amount of combined acid present.

ESTIMATION OF GASTRIC FERMENTS

When free hydrochloric acid is present in stomach contents it is not necessary to estimate for ferments, because pepsin, etc., are almost certain to be present in their normal proportions.

In cases, however, where there is no free acid an estimation for the pepsin content

should be made as we usually find some pepsin ferment present in such cases, unless the case be one of advanced carcinoma or marked atrophy of the gastric mucosa.

The method for estimating two of the ferments will be described.

Pepsin.—This ferment converts acid albumin first into proto- and hetero-albumose, then into deutero-albumose, and finally into peptone. It is present first as the inactive proferment pepsinogen, which is converted by acid into active pepsin.

Qualitative Test.—10cc. of filtered gastric contents are taken in a test tube; to this is added, with shaking, weak hydrochloric acid solution till a reaction is just visible with congo-red test paper. A fragment of hard-boiled white of egg is placed in the fluid, and the test tube with its contents is set aside in an incubator for about an hour. If pepsin be present, digestion of the egg will have taken place wholly or in part.

Hammerschlag's Method of Pepsin Estimation.—Make up an acid albumin solution, viz.:—

Raw white of egg . .	30 cc.
25 per cent. HCl solution	4 "
Tap water . . .	250 "

Pour 10cc. of the above solution into each of two test tubes and to one add 5cc. of filtered gastric contents, to the other add 5cc. distilled water. Then pour the contents of each tube into an Esbach tube up to the mark U. Place both in an incubator for one hour. At the end of that time remove them and add Esbach's solution up to the mark R. Set the tubes aside in a rack for 24 hours and then read off the result on each tube, the difference between the two readings will give the amount of digestion which has taken place. This may be expressed in percentages.

EXAMPLE :—1st tube precipitate reads 2.
2nd tube precipitate reads 4.

Therefore, 4 − 2 = 2 = 50 per cent. of the albumin has been digested.

Normally about 80 per cent. of the albumin is digested in an hour.

N. B.—The albumoses formed during the digestion may be precipitated by the Esbach's reagent, but it has been shown that this precipitate is so fine and comes down with such difficulty as to be a negligible quantity.

This method gives quite satisfactory results in judging of peptic activity.

Rennin Ferment.—This ferment curdles

milk and is supposed by most authorities to vary in quantity in the same ratio as pepsin. Some authorities consider that pepsin and rennin are identical.

The estimation of rennin ferment may be carried out very simply and accurately by means of Willcox's method (*Lancet*, 1908):—

"Narrow test tubes, five inches by three-eighths of an inch, are taken; into each 5 cubic centimetres of fresh unboiled milk are added. They are placed in a water bath at 40° C. Into the tubes are placed *seriatim* gradually increasing quantities of the filtered gastric contents. These are run in from a pipette which is graduated in $\frac{1}{100}$ths of a cubic centimetre. Thus are added 0·01, 0·05, 0·1, 0·15, 0·2, 0·25, 0·3, 0·35, 0·4, 0·5, 0·6, 0·7, 0·8, etc., of gastric contents. The liquids are mixed by gently inverting each tube. The tubes are left 30 minutes in the bath, and then it is found that above one point in the series the contents of the tube are solid and do not flow out on inversion of the tube, while below this point the contents flow out on inversion of the tube.

The minimum quantity of gastric contents to cause complete clotting, so that the contents of the test tube do not flow out on

inversion, gives an accurate measure of the rennin activity."

In the case of a normal adult 0·2cc. gastric contents is the minimum required to coagulate the tube. From this may be calculated the quantity of rennin above or below the normal. If x=the smallest quantity of "contents" required to coagulate the milk, then $\frac{\cdot 2}{x}$ will indicate the amount of rennin above or below the normal, *e.g.*, ·05 was the smallest quantity to cause clotting, then $\frac{\cdot 2}{\cdot 05}$=4 times the quantity of rennin present.

MICROSCOPICAL EXAMINATION OF GASTRIC CONTENTS

The result obtained from this step in the examination of a test meal is disappointing, and in many cases is of little value.

The following are the more commonly seen pathological elements:—

1. Pus cells of various shapes and sizes may be found when there is a chronic inflammatory condition of the wall of the stomach or when tumour growth is present.

2. Blood cells may be present in varying quantity and varying stages of degeneration.

3. Yeasts in the process of active growth may be seen when dilatation is present.

They occur in colonies of rounded cells rather smaller than a blood corpuscle and from which budding processes are formed.

4. Sarcinæ are frequently found in large number in diseased stomachs — especially dilated stomachs. Under the microscope they appear like diminutive bales of cotton. They are not typical in themselves of malignant disease.

5. Micro-organisms of various kinds may be found when HCl is absent, and the emptying of the organ is slow.

The most important organism to look for is the Boas-Oppler bacillus. It is an elongated, thin, non-motile organism occurring in chains, and is irregularly Gram positive. When present in large numbers it is highly suggestive of carcinoma of the stomach.

In addition to these pathological constituents, the food in various stages of digestion is seen under the microscope, *e.g.*, (1) starch granules are easily made out by their concentric rings, and, if digestion has not proceeded too far, they will stain blue with iodine ; (2) fat droplets are easily determined by their shape and size, and they stain orange with Sudan iii ; (3) muscle fibre is recognised by noting its cross striations.

THE FÆCES

The examination of the fæces can only be briefly described.

Three methods of examination are available, viz. :—

1. Macroscopic.
2. Chemical.
3. Microscopic.

Before making any careful estimation of digestive capacity from a fæcal examination it is advisable to keep the patient on a special diet.

Schmidt and Strasburger give two examples for test diets ; the second being suited for quantitative estimations. The average weight of dried fæces got from it would be about $1\frac{3}{4}$ oz. (50 grms).

TEST DIETS PREPARATORY TO FÆCES EXAMINATION

Diet No. 1.—In morning : One pint (1 half litre) of milk, or tea or cocoa made with milk or water ; 1 roll with butter, and 1 soft-boiled egg. Breakfast : One dish of oatmeal cooked with milk and strained, with salt or sugar as desired ; may substitute gruel or porridge. Noon : $\frac{1}{4}$ lb. chopped lean meat,

broiled in butter and underdone; potato purée. Afternoon: Same as morning. Evening: One pint (1 half litre) of milk, 1 roll with butter, 1 or 2 eggs softly boiled.

Diet No. 2.—In morning: One pint (1 half litre) of milk, $1\frac{3}{4}$ oz. (50 grm.) toast. Forenoon: Strained oatmeal prepared from $1\frac{3}{8}$ oz. (40 grm.) oatmeal, $\frac{1}{4}$ oz. (10 grm.) of butter, 7 oz. (200 cc.) milk, $10\frac{1}{2}$ oz. (300 cc.) water, 1 egg and salt. Noon: $3\frac{3}{4}$ oz. (125 grm.) chopped beef broiled with $\frac{1}{2}$ oz. (20 grm.) of butter, $8\frac{1}{2}$ oz. (250 grm.) potato purée prepared from $6\frac{1}{2}$ oz. (190 grm.) potato mashed, $3\frac{1}{2}$ oz. (100 cc.) milk, $\frac{1}{4}$ oz. (10 grm.) butter and salt. Afternoon: Same as morning. Evening: Same as forenoon. The diet is given for three days. They give ·3 grm. of powdered carmine at the commencement of the diet.

The British measures mentioned above are approximately equal to the Continental quantities mentioned by Schmidt & Strasburger.

N.B. The second diet is calculated to contain—

102 grm. of protein.
111 ,, ,, fat.
101 ,, ,, carbohydrate.

It is equal to 2,234 calories.

MACROSCOPIC EXAMINATION

A normal stool consists of undigested food, bacteria in enormous quantities, intestinal secretion, salts, etc,

REACTION.—When tested with litmus paper a normal stool is most frequently faintly alkaline, although it may be neutral or faintly acid. If the patient is on a milk diet the stool may be very acid.

QUANTITY AND FORM.—The quantity passed per diem varies greatly and depends largely on the quantity of food taken. A stool may assume almost any form from a scybalous mass to profuse brownish fluid. In malignant disease, in the lower part of the alimentary canal, the fæces sometimes assume a flattened appearance like ribbons, and the same appearance is produced temporarily by spasms of the large intestine. The stool may be very frothy when intense fermentation is present; and when the pancreatic function is abnormal there is much increase of fat in the stools.

COLOUR.—The normal colour of a stool is due to hydrobilirubin produced by the reduction of bilirubin. Bilirubin appears in the stools of breast-fed infants, and is also seen in cases of diarrhœa when the intestinal contents are hurried along too quickly, pre-

venting the reduction of the bilirubin. Such stools have a yellow or golden yellow colour.

Various kinds of foods give rise to various coloured fæces, *e.g.*, milk gives a light coloured stool, green vegetables give a greenish stool, meat makes the stool darker, etc. Drugs also impart certain colours to the fæces, *e.g.*, bismuth blackens the dejecta, the taking of iron gives a dark colour to stools.

The recognition of blood in the stools is of the utmost importance. Blood in a stool may be readily seen, or it may be of the "occult" type when it can only be detected by chemical means.

Stools may be clay-coloured, due to an increase of fats or complete absence of bile pigment.

The fæces may be mixed with pus, which will give them a lighter colour than usual. If an abscess bursts into the alimentary canal pure pus may be passed per rectum.

ODOUR.—A normal stool emits a characteristic odour brought about by the decomposition taking place in its constituents. Skatol and indol are formed and produce the odour. It is well known that the stools of excessive meat-eaters smell more strongly than those who eat sparingly of meat. In

many debilitated patients it will be found that the stools are very foul-smelling, indicating an excessive putrefactive change in the bowel, which produces a chronic toxæmia of the system.

FOREIGN BODIES AND EXTRANEOUS MATTER.—Many foreign bodies may be passed per rectum which have been taken into the mouth by accident or intent. A gall stone or some other form of concretion may work its way into the alimentary canal. When this is suspected the fæces may be examined by drawing them through two layers of gauze, or by a Boas sieve, which will retain any stone and allow the finer portions of fæces to pass through by the aid of a stream of water from above.

Mucus may be found in a stool, mixed with the fæces or sticking to hard masses. It must not be mistaken for a tape worm. When mucus is present, it is indicative of some diseased or catarrhal condition of the alimentary tract.

Intestinal parasites may be found in a stool, and the head of tape worms may be got by straining the stool through gauze. The smaller parasites and eggs can only be detected by the microscope.

BLOOD IN THE STOOLS.—The following types of sanguineous stools may be noted :—

1. A normal stool may have bright blood over or around it. This appearance would indicate blood in the lower part of the alimentary tract—most commonly from the anal canal.

2. Solid fæces may be passed with dark blood throughout. This would indicate a hæmorrhage from the upper part of the alimentary tract.

3. A liquid stool may be mixed with blood, and in this case the colour of the blood will give some indication of the part from where the blood has come. The darker the blood the farther up the alimentary tract the hæmorrhage will be.

4. Black or tarry stools may result from gastric hæmorrhage, and may be present without any hæmatemesis. A hæmorrhage from a duodenal ulcer may produce a somewhat similar appearance of the fæces.

5. In typhoid fever an intestinal hæmorrhage will show blood in the stool. The colour will be fairly bright, because the hæmorrhage has probably taken place low down in the ileum, and defæcation has rapidly followed.

6. In cases of intussusception blood may be passed with serous matter associated with diarrhœa.

7. Finally, it is worth while remembering that a patient suffering from gastric symptoms may pass a slightly darker stool than normal, which contains occult blood.

CHEMICAL EXAMINATION

Tests for Occult Blood in the Fæces.

1. ALOIN TEST :—

Precautions.—(1) For two or three days stop all foods containing hæmoglobin or green colouring matter, and all drugs; (2) Diet.—Milk, bread, eggs, and fruit.

Method.—A portion of a dark coloured stool should have the urobilin removed by extraction with alcohol before carrying out the test.

Part of the stool is dried with filter paper, and 5 grms. are mixed thoroughly with 5cc. of glacial acetic acid. The fat present is extracted with 10cc. ether. About 1cc. of oxygenated turpentine or ozonic ether is carefully layered over the acetic acid extract, and to this is added a fresh 3 per cent. alcoholic aloin solution. If blood be present, a fine red line develops in from 3–5 minutes at the line of separation.

2. BENZIDINE is a grey crystaline powder, soluble in alcohol, and in a slightly acid solution will turn a purplish colour in the presence of blood and hydrogen peroxide. Oxidising enzymes will produce a similar colour reaction which boiling will, however, destroy. Merk issues specially prepared benzidine of standard strength for this test.

Test.—A portion of the fæces about the size of a small hazel nut is thoroughly mixed with water and boiled. A fresh alcoholic solution of benzidine (Merk) is made up for each set of tests. A knife point of benzidine is dissolved in 2–3cc. of glacial acetic acid. 10-12 drops of this solution are mixed with about 3cc. of hydrogen peroxide. No colour reaction should take place if the test tube and reagents are clean. A few drops of the solution of fæces are added to the above mixture, and if blood be present a green or blue colour will develop in from 2-3 minutes. This may ultimately become violet.

Other tests for occult blood have been described, but the two already mentioned are sufficiently accurate.

HYDROBILIRUBIN AND BILIRUBIN.—These substances may be detected by mixing a small portion of the fæces with perchloride

of mercury. The mixture is set aside for twenty-four hours. At the end of this time particles of fæces stained with hydrobilirubin appear red and those stained with bilirubin are green in colour. When bilirubin is present there is probably catarrh of the bile ducts.

MICROSCOPICAL EXAMINATION

PREPARATION OF SLIDE.—If the fæces are fluid a drop or two is put on a slide from a pipette, care being taken to include any possible pathological material in the stool. The drop is spread by placing a cover slip above it and gently pressing the two together.

If the fæces be solid a small portion is removed on the end of a match and placed on an ordinary microscope slide. Another slide is placed on the top and the fæcal matter squeezed out into a thin film between the two slides.

Most useful information is gained by using a low power of the microscope.

PROTEID will show itself as incompletely digested muscle fibres. The state of indistinctness of the cross striations may be taken as a guide to the extent of proteid

digestion. If intestinal digestion is complete no muscle fibres should be seen. If there are numerous bundles of muscle fibres with well-marked striations then digestion of proteid has been faulty.

Connective and elastic tissue fibres may be seen somewhat like mucus but possessing longitudinal striations. The presence of a large quantity indicates disturbance of gastric function as pancreatic and intestinal digestion has little effect on connective tissue.

CARBOHYDRATES. — Undigested starches are seldom found in the fæces of a patient on an ordinary mixed diet. When present they may be detected by staining with iodine. In this way they show up as indigo-like grains. When too much undigested starch is present, fermentation of the fæces takes place and they are markedly acid.

In diseased conditions of the pancreas the digestion of starch will be carried on by the bacteria present in the alimentary tract.

FAT. — In normal stools we find 6–10 per cent. of fat present. In diseased conditions of the pancreas the amount may rise to 85 per cent. of the total stool.

Fat occurs in the fæces as—

1. Fatty acids.
2. Soaps.
3. Neutral fats.

FATTY ACIDS may be detected as short, delicate curved needles, difficult to make out. They are soluble in ether and on warming.

SOAPS appear as long needles arranged in clusters or fans. They are insoluble in ether and on warming.

NEUTRAL FATS are seen as droplets which are soluble in ether.

These products of digestion may be demonstrated by preparing two films on microscope slides, and staining one with saturated alcholic Sudan iii, and the other with dilute carbol-fuchsin. Neutral fats stain orange red with Sudan iii. Fatty acid crystals stain orange red with Sudan iii. and brilliant red with dilute fuchsin. Soap-crystals stain a dull red with fuchsin and do not stain with Sudan iii. Bacteria stain in the same way. If one wishes to prove that the soap crystals are stained, then glacial acetic acid must be added and heat applied. If droplets have increased in number this indicates presence of soaps

which have been changed to fatty acids and may be stained as such.

Various other substances may be found in the fæces such as vegetable tissues, crystals of triple phosphates and oxalate of lime, epithelial and pus cells. These may be stained by Leishman's reagent.

Intestinal parasites of many kinds may be found in the fæces. These are fully detailed in various books of pathology and cannot be described here.

Bacteria of various kinds are plentiful in the fæces.

Tubercle bacilli are frequently found in patients suffering from pulmonary or abdominal tuberculosis. They may be demonstrated by using the antiformin method of separation.

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