Demonstrations in physiological & pathological chemistry : with a concise account of the clinical examination of urine / by Charles Henry Ralfe.

DEMONSTRATIONS IN PHYSIOLOGICAL & PATHOLOGICAL CHEMISTRY

RALFE

DEMONSTRATIONS IN

PHYSIOLOGICAL & PATHOLOGICAL CHEMISTRY,

WITH A CONCISE ACCOUNT OF THE CLINICAL EXAMINATION OF URINE.

BY

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PREFACE.

EXAMINATIONS in Physiology of late years have assumed a practical character. It is not sufficient now for a student to exhibit a general knowledge of the subject, acquired solely from a course of routine lectures and the perusal of a text-book, but he must prove that he has made observations for himself. He is expected to be able to prepare for microscopic examination the tissues and organs of the body, to test and separate the proximate principles and the chief products of decomposition; and to demonstrate the chemical composition and action of the secretions and the chief constituents of the tissues and nutritive fluids. In addition, he is expected to make himself practically acquainted with the chief physiological instruments and the methods of employing them.

In order to meet the increased requirements of the student in this respect, the ordinary course of lectures on Physiology has been supplemented by practical Demonstrations in most of the medical schools, and as far as Histology is concerned, the ordinary student has a sufficient choice of hand-books to direct him in his practical study. With Physiological Chemistry, however, the case is different; for no text-book suitable to the requirements of the ordinary student has yet appeared, and demonstrators as a rule have made for themselves a synopsis of the characteristic reactions of the chief constituents of the tissues and secretions from the exhaustive and admirable treatise arranged by Klein, Foster, Burdon-Sanderson and Brunton, for the Physiological Laboratory and from German text-books.

The present volume took its origin in this way: On taking charge of the class in Physiological Chemistry at St. George's Hospital, six years ago, I wrote out a series of elementary papers giving the important chemical and physical characters and reactions of the proximate principles; products of decomposition such as urea and uric acid; the composition and action of the digestive fluids; together with

the characteristic reactions of blood, milk, &c. At each Demonstration the student had one of these papers given to him, and he worked through the reactions set down in it. In addition, however, to these elementary papers, I have added some of the more elaborate processes and reactions, in order to meet the requirements of more advanced students and those preparing for the examinations at the Universities of Oxford, Cambridge, and London, or for the Fellowship of the Royal College of Surgeons of London. These processes and reactions are distinguished by an asterisk.

LONDON, *August*, 1880.

"Chymia egregia ancilla medicinæ, non alia pejor domina."

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IN

PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY.

PROXIMATE PRINCIPLES.

DEMONSTRATION I.

THE SACCHARINE AND AMYLACEOUS PRINCIPLES.

Amylaceous.		Saccharine.	
Starch.	$C_6H_{10}O_5$	Glucose.	$C_6H_{12}O_6$
Glycogen.	$C_6H_{10}O_5$	Lactose.	$C_{12}H_{24}O_{12}$
		Inosite.	$C_6H_{12}O_6$

These substances are also termed *Carbo-hydrates*, from the fact of their containing Hydrogen and Oxygen in proportion to form water, united with Carbon. Owing to this composition they are readily converted the one into the other by the removal or addition of the elements of water; thus,

Starch. Glucose.

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$$

The carbo-hydrates closely resemble one another in their chemical characters. They are neutral in their

reaction, and have little disposition to enter into combination. They all have a strong action on polarized light.

The saccharine principles (with the exception of inosite) reduce alkaline copper solutions, throwing down the cuprous oxide. Boiled with liquor potassæ, they give a brown colour to the solution. And their solutions undergo vinous fermentation when yeast is added.

The amylaceous principles are converted into the saccharine by the action of dilute acids, and by the salivary, pancreatic, and intestinal juices ; treated with free iodine they form coloured compounds, that of starch yielding a characteristic deep blue colour, and glycogen a violet or maroon red coloration.

(1) STARCH. $C_6H_{10}O_5$.

Starch is one of the chief constituents of our food, but being very insoluble it is converted into grape sugar by the action of the salivary, pancreatic, and intestinal juices to allow of its absorption from the intestinal canal. In certain parts of the body, as in the prostate, in the ependyma of the ventricles, the fornix, the choroid plexus, the retina, and spinal cord, starch granules, the "corpora amylacea" of Kolliker and Purkinje, are sometimes found in advanced age.

Chemical and physical characters occur in small rounded granules of irregular form, marked with concentric laminæ, and having a pore, "the hilum," at one spot on its surface. Starch is insoluble in cold water, but on being boiled it swells up, the granules burst and form a stiff paste.

REACTIONS.—Make a solution of starch by adding an extremely small quantity of starch (1 part of starch to 25

of water) to a test-tube three parts full of cold distilled water. Notice that the starch is not dissolved. Heat the upper part of the tube; the milky turbidity gradually clears up as the boiling point is reached, and the fluid assumes a gelatinous appearance. Now boil the whole contents of the tube. Set aside to cool; when cold

Test 1. *The solution is neutral;* it gives no reaction with blue or red litmus-paper.

Test 2. *It gives a blue colour with free iodine.*—Place a few drops of the solution of starch in a test-tube, add a drop of aqueous solution of iodine, a deep blue colour will be developed. Heat, the blue colour disappears, but is again partially developed as the fluid cools.

Test 3. *By boiling with dilute acids it is converted into glucose* (grape sugar).—To about a drachm of the starch solution add 10 drops of dilute sulphuric acid, boil for some minutes.[1] Notice the solution loses its mucilaginous consistence and becomes thin and limpid. Divide into two portions (*a*—*b*), test when cold—

(*a*) gives no reaction with iodine, showing disappearance of starch;

(*b*) gives a reddish yellow precipitate with a warm alkaline solution of cupric sulphate; showing conversion of starch into glucose (*vide* Glucose, § 3, Test 5).

Test 4. *Saliva converts it into glucose.*—Add a small quantity of saliva to half a test-tube of solution of starch and place in water-bath at 40° C. for ten minutes. Divide into two portions (*a*—*b*), test with iodine and warm alkaline solution of copper as in Test 3. The result will be the same.

(2) Glycogen. $C_6H_{10}O_5$ (syn. *Animal starch*, amyloid matter).

[1] If this change is not developed the solution must be boiled a little longer. Dextrin is formed at the same time as glucose. The decomposition that occurs may be represented as

$$\overset{\text{Starch.}}{3\,(C_6H_{10}O_5)} + H_2O = \overset{\text{Glucose.}}{C_6H_{12}O_6} + 2\,\overset{\text{Dextrin.}}{(C_6H_{10}O_5)}$$

Glycogen is found in the tissue of muscle and liver, also in the placenta and embryonic tissues of all animals. It is most probably derived chiefly from the carbo-hydrates introduced into the body with the food, since the largest amount of glycogen is obtained from the liver after a diet formed exclusively of these substances. At the same time there can be little doubt that the decomposition of albuminoid substances, which are broken up in the hepatic cells into certain nitrogenous products on the one hand, and into non-nitrogenous products on the other, furnish among the latter class a proportion of the glycogen met with in the liver. This supposition is rendered more probable since the liver of animals fed exclusively on meat still yields a considerable quantity of glycogen.

Glycogen is also largely formed in muscular tissue, probably as one of the products of the decomposition of the albuminous constituents. By its own decomposition it probably furnishes a supply of force, which helps to maintain the energy of the muscular contractions, since muscular action is associated with a marked diminution in the amount of glycogen in muscular tissue.

Opinions are divided among physiologists as to the final changes glycogen undergoes in the body. According to Claude Bernard the glycogen formed in the liver is converted into sugar immediately after its production, and in this state is carried off by the hepatic vein into the system, where it is destroyed by oxidation in the peripheral capillaries. Dr. Pavy, on

the other hand, contends that the liver does not transmit sugar to the circulation, but that it exerts an active influence in preventing this principle reaching it to any material extent. In other words, according to Dr. Pavy, the liver is a sugar-assimilating, not a sugar-forming organ. He believes that the sugar derived either directly or indirectly from the food, and absorbed from the alimentary canal, is under normal circumstances stopped by the selective or secreting action of the cells of the liver, and in these transformed into glycogen. And as this body belongs to the class of non-diffusible bodies, it possesses the properties which physically contribute to its retention in the hepatic cells, where he suggests it undergoes a change which forms one of the links in the series leading up to the final issue—the utilisation of sugar as a force-producing agent in the system. (See Glucose, § 3.)

Preparation. (Brücke's method.)—The liver taken from an animal in full digestion just killed (the liver from an oyster just opened is well suited for the purpose) is rapidly comminuted and thrown into boiling water for a few minutes, the coagulated mass is then removed, drained, and triturated in a mortar, and boiled for a quarter of an hour; the solid mass is then removed by filtration. The filtrate is then concentrated, and set aside to cool; and when cold, a drop of hydrochloric acid and a drop of potassio-mercuric iodide solution is alternately added, stirred thoroughly for five minutes and filtered. To the filtrate alcohol is added in minute quantities till a precipitate appears, when no more alcohol is to be added lest other substances be precipitated. The precipitate is then removed to a weighed filter and washed with dilute alcohol (60 per cent.), and afterwards with glacial acetic acid; it is then to be dried and weighed.

Chemical and physical properties.—Glycogen is a yellowish white, amorphous, substance; soluble in water, insoluble in alcohol. With iodine it gives a violet or maroon red coloration. Like vegetable starch, boiled with dilute hydrochloric acid, it is converted into dextrin and glucose; mixed with saliva at temperatures of 36° C., it is converted into glucose. It does not reduce copper salts from their alkaline solutions.

(3) GLUCOSE. $C_6H_{12}O_6$ (syn. *Grape sugar*).

Grape sugar is present as a normal constituent in healthy blood, it can always be obtained from the liver after death; it is also found in fœtal urine, and in the fluid contents of the amnion and allantois. It is present in extremely minute quantities in normal adult urine. In the disease known as diabetes a large quantity is always found in the urine.

Sugar is introduced into the economy with the food; either directly with the saccharine principles, or indirectly by the transformation of the starchy matters by the action of the salivary, pancreatic, and intestinal secretions. Sugar also is formed in the organism itself by the conversion of *glycogen*, a principle analogous to starch, found abundantly in muscular and liver tissue. (See Glycogen, § 2.)

Sugar fulfils the following purposes in the economy: 1. By its decomposition into carbonic acid and water it furnishes a certain quantity of heat which aids in maintaining the temperature of the body. 2. Before its ultimate conversion into these products is completed, it furnishes by its oxidation a number of fatty acids of which the most important is lactic acid. These acids set free in the circulation are essential for the

performance of the functions of the body; for without them no acid salts could be formed, nor would there be sufficient acid generated for the digestion of albuminous substances. 3. Sugar also is converted into fat in the body, the fatty acids formed by its oxidation probably uniting with glycerin to form the neutral fats or glycerides.

Chemical and physical properties.—Glucose is sold in irregular warty masses, which under the microscope exhibit rhombic tablets.

Test 1. *It is extremely soluble in cold water*, and the solution has a faintly sweet mawkish taste.

Test 2. *The solution is neutral*, it gives no reaction with blue or red litmus-paper.

Test 3. *Alkalies, when heated with a solution of sugar, give a dark brown colour.*—Boil a small quantity of solution of glucose with an equal amount of liquor potassæ; the solution will acquire a brownish tint (*Moore's test*).

Test 4. *Yeast added to a solution of sugar induces vinous fermentation.*—Nearly fill a tall urine-glass with a solution of grape sugar. Take the specific gravity of the solution with a hydrometer. Record the specific gravity on a slip of paper. Now add a fragment of yeast (size of a pea), and put the glass and its contents aside for twenty-four hours near the fireplace. At the next demonstration, or in twenty-four hours' time, take the specific gravity again. It will be found to have fallen, because vinous fermentation has been set up, and the sugar converted into carbonic acid and water. The difference of each degree of specific gravity lost is equivalent to one grain of sugar in every fluid ounce of the solution.

Test 5. *Sugar reduces alkaline copper solutions.*—Fill a test-tube quarter full of solution of glucose, add a few drops of dilute *cupric* sulphate solution, and then add liquor potassæ till the test-tube is half full. Heat gently, a yellowish red precipitate of *cuprous* oxide will fall (*Trommer's test*). If, in addition to the copper salt and liquor potassæ, sodæ potassio-tartrate be used, then test solution is called the alkaline tartrate solution (*Fehling's*

test), which also reduces the *cupric* salt to a *cuprous*. (N.B.—As other substances besides sugar have a reducing action on copper, it is advisable to confirm with tests 3 and 4).

PRACTICAL APPLICATIONS. *(*a*) *To ascertain the presence of sugar in blood.*—Agitate blood with four times its bulk of alcohol, allow the mixture to stand, and then filter; extract the residue with a little hot water and rub it thoroughly in a mortar with animal charcoal, add a little more hot water, and filter. Add the aqueous to the alcoholic filtrate, and evaporate to near dryness over a water bath. Apply tests 3, 4, and 5.

*(*b*) *To ascertain the presence of sugar in liver tissue.*—Wash out the liver of a recently killed animal by passing a stream of water, injected by means of a syringe, through the portal vein. The fluid issuing from the hepatic veins will be found by tests 3 and 5 to contain sugar, the quantity diminishing as the washing out is proceeded with, till no reaction with tests is attained. If after the sugar has been washed out, the liver be laid aside for an hour or so, and the washing again performed, a small quantity of sugar will be detected, showing that sugar has been formed by the liver-cells since the removal of the first portion.

(*c*) *To determine the quantity of sugar present in urine.* (*vide* Urine.)

(4) LACTOSE. $C_6H_{12}O_6$ (syn. *Milk sugar*), is a characteristic constituent of milk. It is found in no other secretion. The large quantity always present in milk is an evidence of the important part which sugar takes in the nutrition of the young mammal.

Chemical properties.—Like glucose it reduces copper salts from their alkaline solutions. It differs from glucose in not readily undergoing vinous fermentation when yeast is added. Boiled for some hours with dilute acids it forms *galactose*, a sugar isomeric with glucose, and like it, ferments readily on the addition of yeast. (For estimation of lactose, see Milk.)

(5) INOSITE. $C_6H_{12}O_6$ (syn. *Muscle sugar*) was originally discovered by Scherer in the muscular substance of the heart. It is a constant constituent of voluntary muscular fibre, and is also found in the tissues of the kidney, liver, spleen, brain, and testicle. In certain diseases, as Diabetes, Bright's disease, Phthisis, Syphilitic cachexia, and Typhus, it is met with in the urine.

* *To obtain inosite from urine.*—The urine to be tested for inosite is completely precipitated with sugar of lead, filtered, and the warm filtrate treated with basic acetate of lead as long as any precipitate is formed. It is better that the urine should be concentrated to one-fourth before it is precipitated. The lead-precipitate collected after twelve hours' standing is washed, suspended in water, and then decomposed by sulphuretted hydrogen. After the filtrate has been left at rest a short time, a small quantity of uric acid separates from it; this is removed by filtration, and the fluid so concentrated as to remain permanently turbid when treated with an equal volume of alcohol. It is then heated until the turbidity disappears, and allowed to stand one or two days. The crystalline mass thus obtained is purified by re-crystallisation, and then subjected to the tests of nitric acid, ammonia, and chloride of calcium, as well as of tartrate of copper (Neubauer and Vogel).

Chemical properties.—Although inosite is isomeric with glucose, it presents none of the characteristic reactions of the group; thus it does not reduce the copper salts[1] from their alkaline solutions, nor does it undergo vinous fermentation with yeast, nor has it any action on polarized light.

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[1] Although no reduction of copper takes place a green solution results, from which, after a time, a flocculent, greenish precipitate is separated, the upper part of the fluid becoming blue. If the precipitate is separated by filtration, and the filtrate again boiled, the same play of colour will be again observed. This reaction is often observed in urine.

The following is a characteristic and delicate test for inosite : a small quantity of the fluid containing inosite is evaporated to dryness on a piece of platinum foil with a drop or two of nitric acid, the residue moistened with ammonia and calcium chloride, which on evaporation yields a beautiful rose tint.

Requirements for Demonstration I.

MATERIALS.—Powdered starch. Yeast. Grape sugar (Honey). Oysters.

REAGENTS.—Solutions of iodine : cupric sulphate : sodium potassio-tartrate. Liquor potassæ. Dilute sulphuric acid. Red and blue litmus-paper.

APPARATUS.—Test-tubes. Water bath. Spirit-lamp or Bunsen-burner. Hydrometer. Urine-glass. Thermometer.

DEMONSTRATION II.

THE FATTY PRINCIPLES.

Saponifiable.		Non-Saponifiable.	
Palmitin.	$C_{51}H_{98}O_6$	Cholesterin.	$C_{26}H_{44}O$
Stearin.	$C_{57}H_{110}O_6$	Serolin.	?
Olein.	$C_{57}H_{104}O_6$	Stercorin.	?
		Excretin.	?

I. *The saponifiable or neutral fats* are formed by the union of a fatty acid radical with glycerin; thus, stearin consists of three parts of the acid radical, stearyl $C_{18}H_{35}O$, which has replaced three atoms of the typical hydrogen from $\left.\begin{matrix}C_3H_5\\H_3\end{matrix}\right\}O_3$, glycerin, to form the new substance $\left.\begin{matrix}C_3H_5\\3(C_{18}H_{35}O)\end{matrix}\right\}O_3$, or stearin; and in a similar manner the radicals of palmitic and oleic acid unite with glycerin to form palmitin, $\left.\begin{matrix}C_3H_5\\3\,(C_{16}H_{31}O)\end{matrix}\right\}O_3$, and olein, $\left.\begin{matrix}C_3H_5\\3(C_{18}H_{33}O\end{matrix}\right\}O_3$.

(6) Palmitin, $C_{51}H_{98}O_6$. This substance is obtained from human fat by separating it from the stearin with which it is combined, by melting in a

water bath, temp. 62°·8 C., and extracting with ether at this temperature. The etherial solution on evaporation deposits palmitin.

(7) STEARIN, $C_{57}H_{110}O_6$. Is obtained from human fat after the removal of the palmitin by raising the temperature of the water bath to 69°·7 C. adding an equal quantity of ether. The etherial solution being decanted on cooling deposits stearin.

(8) MARGARIN consists or 10 per cent. of stearin and 90 per cent. of palmitin. Margarin is obtained by heating fat in a water bath, and stirring with an equal quantity of alcohol for some time; the alcoholic solution is then filtered off, and on cooling it will deposit delicate needle-shaped crystals, which arrange themselves in whorled groups or feathers. The melting-point of margarin is 47°·8 C., which is considerably lower than the melting-point of its constituent components.

(9) OLEIN, $C_{57}H_{104}O_6$. Olein can be obtained tolerably pure by heating fat in a flask, and filtering it when cold; the filtered solution is concentrated by evaporation, and by the addition of water the olein is separated; the product is then exposed to cold at 0° C., and the solid portion submitted to pressure; the liquid that separates is almost pure olein.

Human fat is formed of a mixture of stearin, palmitin, and olein; the two former constitute about three-fourths of the fat of the body and form the solid portion; whilst the olein represents the remaining fourth, and is the liquid or oily constituent.

Fat is found in all the tissues and fluids of the body, usually forming distinct masses or globules, which do not combine with the other elements of the body, but remain free, either suspended in fluids, or lodged between fibres, or deposited in cells.

Fat is essential to the growth and nutrition of the tissues, a larger proportion of fat being met with wherever cell growth is going on rapidly, that in tissues which are fully developed. Fat by its combustion in the economy furnishes a quantity of force to maintain the energy, and heat to supply the temperature of the body; indeed fat may be regarded as the storehouse of carbon; and one apparent advantage of its freedom from combination with other elements is that it is always ready for immediate service, whenever the requirements of the system demand it.

The chief source of the fat of the tissues is of course from the oleaginous constituents of the food: but fat is also formed by the decomposition of the saccharine and albuminous principles, which yield fatty acids, and which, probably combining with glycerin, are converted into fat before their ultimate reduction to carbonic acid and water.

Fat is found in only extremely minute quantities in the healthy human excretions, since in the body it is always decomposed into carbonic acid and water, and in this form passes out of the economy. In certain diseases however fat appears in the excretions. In cases of occlusion, for instance, of the pancreatic and biliary ducts the fats introduced with the food

into the intestinal canal are not emulsionized and saponified, and consequently are not absorbed, but pass unaltered out of the system with the fæces. Again, in chyluria, fat globules are met with in the urine.

Whenever the process of oxidation is impeded or imperfectly performed, we find that fat accumulates in the organs and tissues; for example, we find it in the fatty degeneration of the liver and voluntary muscular fibre met with in all cases of phthisis, or pulmonary diseases which have run a chronic course; and in obesity, the penalty of sedentary or self-indulgent habits. Whenever the supply of blood is cut off from a part, or its flow diminished, the oxidation of that part is of course arrested, and, as a consequence, fatty degeneration occurs; for instance, a thrombus blocks up a cerebral artery, and acute softening of the cerebral substance supplied by that artery is the result. And even if the supply be only diminished instead of entirely arrested, the result is the same only not so rapid. If an organ or member is long disused, or its functional activity impaired, it undergoes fatty degeneration, since the physiological stimulus being no longer supplied, the same quantity of blood does not circulate through the part as when it was in full activity and vigour; the fatty degeneration of the muscular fibres of the uterus after delivery, and the tendency to accumulate fat after the active work of life is over, illustrate this point.

Certain poisons, as the salts of the bile acids,

phosphorus, &c., when introduced into the system produce rapid fatty degeneration of the organs and tissues, by causing the destruction of the blood corpuscles and the consequent diminution of the oxidizing power of the blood.

Chemical and physical properties—

Test 1. *They are insoluble in cold water.*—Shake up a little olive oil with water in a test-tube. The oil becomes broken up into small globules, which readily reunite and float on the surface of the water.

Test 2. *Readily soluble in ether; benzol, fluid oils, chloroform, and hot alcohol.*—Place a drop of olive oil on a glass slide, add a drop or two of ether, the oil is dissolved; on evaporating the ether with the breath the oil is left as a greasy residue on the glass slide.

Test 3. *They are highly inflammable.*—Burn a small portion of fat on a piece of platinum foil, it flares away with great intensity, leaving little or no residue.

Test 4. *They form an emulsion with a solution of albumin.*—Shake up a small quantity of olive oil with solution of albumin. A milky fluid is formed, this under the microscope will be seen to consist of minute globules. On standing, these globules slowly unite, and form larger ones, and finally, a considerable portion of the oil will separate itself and float free on the surface of the solution of albumin.

Test 5. *Heated with alkalies they are saponified.*—Shake up some olive oil with an equal quantity of liquor potassæ and heat, a soapy fluid, which lathers on agitation, results. (*Saponification* consists in the decomposition of the fat or oil into fatty acid and glycerine. The fatty acid unites with the alkali to form a soap whilst the glycerine remains in solution.)

II. *The non-saponifiable* fatty matters are distinguished from the preceding by not being decomposed or saponified when treated with alkaline solutions. Consequently they can be separated from the other

fats by adding a solution of caustic potash to the etherial solution, which causes the saponifiable fats to dissolve out, leaving the non-saponifiable in solution.

(10) CHOLESTERIN, $C_{26}H_{44}O$. Is the only member of the group which requires particular notice. This substance is generally regarded as an excretory product formed in the substance of the brain and nervous tissue, whence it is absorbed by the blood and carried to the liver; here it is separated from the blood and discharged with the bile into the intestines where it undergoes decomposition.

Cholesterin can be obtained from nearly all the tissues and fluids of the body; it also occurs in several morbid products, as gall-stones, the fluid of hydatid, ovarian cysts, &c.

Preparation.—The fluid or tissue from which cholesterin is to be extracted, must be thoroughly exhausted with ether. The etherial solution is then filtered, agitated with a solution of caustic potash to remove the saponifiable fats. After standing a few minutes the etherial solution containing the cholesterin will separate from the solution of caustic potash. The etherial solution is then drawn off by means of a pipette, and placed in a glass flask and evaporated over boiling water. (N.B.—Care must be taken not to bring the flask near the gas-burner, or the vapour of the ether will take fire. It is advisable to bring the temperature of the water to boiling-point, then withdraw the flame, and plunge the flask up to its neck in the water. When the temperature of the water has fallen to 40° C., remove the flask and replace the burner, and raise the temperature of the water to boiling-point again, then remove the burner and replace the flask, and so on till the whole is evaporated.) Add to the residue left at the bottom of the flask just so much boiling alcohol as will dissolve it.

The alcoholic solution on cooling deposits cholestearin in glistening crystalline plates.

Chemical and physical characters.—Cholestearin, as obtained by the above process, is a white crystalline substance, somewhat resembling spermaceti.

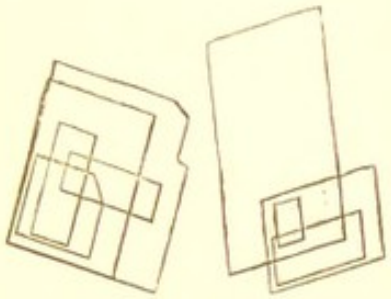

FIG. 1.—Cholestearin.

Test 1. *It is lighter than water, and floats on the surface; and it is not dissolved by it.*

Test 2. *Very soluble in ether.*—Place a minute fragment of cholestearin on a glass slide, and touch it with ether, it is at once dissolved. Evaporate the etherial solution with the breath, and the cholestearin is deposited.

Test 3. *Red coloration given when evaporated with nitric acid, and the residue touched with ammonia.*—Place a minute fragment on a white porcelain dish, add a drop of nitric acid, evaporate to dryness, touch the dried residue with a drop of ammonia, a deep red-brown coloration is developed.

Test 4. *Violet coloration with hydrochloric acid and ferric chloride.*—Place a minute fragment of cholestearin on a white porcelain dish, and add two or three drops of strong hydrochloric acid and one or two drops of dilute solution of ferric chloride, evaporate gently, the residue acquires a beautiful violet colour.

Requisites for Demonstration II.

MATERIALS.—Mutton suet. Olive oil. Gall stones or etherial extract of brain-pulp (made by digesting ox-brains in ether). Solution of Albumin.

REAGENTS.—Distilled water. Alcohol. Ether. Nitric acid. Hydrochloric acid. Solutions of Ammonia. Potassium hydrate. Ferric chloride.

APPARATUS.—Thermometer. Water bath Pipette. Small glass flask. Glass slide. Stirring-rod. Test-tubes. Platinum foil.

DEMONSTRATION III.

ALBUMINOUS AND ALLIED PRINCIPLES.

- **Albumins**
  - Ovo- / Sero- } Albumin.
  - Globulin.
  - Myosin.
  - Fibrin.
- **Modified Albumins**
  - Lardacein.
  - Casein (Alkali albumin).
  - Syntonin (Acid albumin).
  - Coagulated Albumin.
  - Peptones.
- **Gelatins**
  - Mucin.
  - Gelatin.
  - Chondrin.
  - Elasticin.

The albuminoids, or, as they are frequently called, the proteids, are the most important of the proximate principles, constituting as they do the nutritive element, and the basis of all the tissues and fluids of the body. Thus they are found in white of egg, in the blood, in lymph, in chyle, in milk, and in the

juices of the parenchymatous tissues. The albumin of the economy is derived from the albuminoid constituents of the food; as, casein from milk, gluten from bread, myosin from flesh, and ovo-albumin from white of egg. These substances, though differing from each other in many respects, are by the action of the acid gastric juice and alkaline pancreatic juice speedily reduced and converted into peptones, in which form the distinctive characters of the different substances are lost. The peptones are absorbed into the circulation, and again undergo metamorphosis into fibrin, globulin, myosin, casein, &c.

These substances in fulfilling their purpose in the economy break up and become oxidised; on the one hand, into certain saccharine and fatty bodies, which are ultimately eliminated in the form of carbonic acid and water; and on the other, into nitrogenous substances, leucin, tyrosin, uric acid, &c., which are finally eliminated by the kidneys in the form of urea.

All *nitrogenous* substances found in the body belong to this group of proximate principles, or are products derived from their decomposition.

They all contain Carbon, Hydrogen, Nitrogen, and Oxygen, and most of them Sulphur and Phosphorus. The proportions of these elements vary but slightly in the different bodies; the exact constitution of the albuminoids has not however been determined.

A.—CHEMICAL AND PHYSICAL CHARACTERS OF THE GROUP.

The albuminoids are amorphous bodies, and have never been obtained in a crystalline form. *Prepare a solution of*

albumin by carefully separating the white of egg from the yolk. Break up the white by snipping it through several times with scissors. Agitate briskly with four ounces of distilled water, and filter.

Test 1. *Solutions of albumin possess extremely low diffusive powers.*—Notice that in filtering, the solution passes very slowly through the filter-papers; so that several filters have to be used to obtain in a short space of time a quantity sufficient for testing.

Test 2. *Xanthoproteic reaction.*—To a few drops of solution of albumin add a drop of concentrated nitric acid, heat; a yellow colour is developed. A drop or two of ammonia added by means of a stirring-rod, and the solution acquires an orange colour.

Test 3. *Millons reaction.*—Add a few drops of mercuric nitrate to a small quantity of solution of albumin. On boiling, the coagulated albumin acquires a red colour and the fluid becomes pinkish.

Test 4. *Alkaline solution of copper reaction.*—To a small quantity of solution of albumin add an equal bulk of liquor potassæ and two or three drops of cupric sulphate solution, the mixture will assume a violet colour.

Test 5. *Reaction with ferrocyanide of potassium and acetic acid.*—Add a few drops of strong acetic acid to a solution of albumin, a drop of solution of ferrocyanide of potassium will cause a white precipitate. (N.B.—This test distinguishes the albuminoid principles from the gelatinous.)

B.—DISTINCTIVE CHEMICAL AND PHYSICAL CHARACTERS OF THE ALBUMINOIDS.

I. Albumins.

(11) Soluble albumin, as obtained from blood serum or white of egg, is a neutral, viscid, glairy substance. It is met with in all the animal tissues and fluids.

Test 1. *It is soluble in water.*—Shake up a little white of egg, which has been well broken up, with distilled water; notice that it is dissolved. Its solubility distinguishes it from all the other members of the group except the peptones.

Test 2. *It is insoluble in alcohol and ether.*

Test 3. *It is coagulated by heat.*—Place a small thermometer in a test tube and fill the test-tube half full with solution of albumin ; then plunge it in a water bath and gradually raise the temperature. A white film will appear in the solution when the temperature reaches 70° C. (158° F.). (N.B.—The presence of *extremely* small quantities of dilute acids will cause coagulation to take place at a lower temperature ; so also will neutral salts such as sodium chloide and sodium sulphate. On the other hand, an extremely minute quantity of sodium carbonate retards coagulation till a higher temperature is reached.) [1]

Test 4. *It is coagulated by strong mineral acids.*—Place a small quantity of albumin in a test-tube and run a drop of strong nitric acid down the side of the tube to the bottom ; a white zone of coagulated albumin will appear at the point of junction of the two fluids.

Test 5. *It is not coagulated by acetic, tartaric, carbonic, or nomal phosphoric acids.*

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[1] Albumin, even after *dialysis*, always contains certain saline substances, which enter intimately into its composition ; these substances probably are of use in keeping albumin in a state of solution in the body. The point at which the various soluble albumins coagulate seems to depend on the quantity of saline ingredients that enter into their composition.

Ovo-albumin is distinguished from sero-albumin by the following characteristic. The specific rotatory power of ovo-albumin for yellow light is —35°·5 ; that of sero-albumin,—56°. Ovo-albumin is coagulated by ether ; sero-albumin is not. Strong hydrochloric acid readily coagulates ovo-albumin ; and the coagulum is not easily redissolved ; sero-albumin does not coagulate so easily, and the coagulum is more readily redissolved.

Met-albumin ; a modification of albumin met with in dropsical fluids. It is not coagulable by heat ; gives no precipitate with acetic acid or acetic acid and potassium ferrocyanide ; heated with acetic acid, a slight cloudiness is given to the solution ; alcohol precipitates it, but does not coagulate it.

Par-albumin. This substance was obtained by Scherer from the fluid of certain ovarian cysts ; and is usually associated with a body resembling glycogen, and which is capable of conversion into sugar. Its solutions are extremely viscid ; it is precipitated from its warm solutions by acetic acid and carbonic acid gas ; it also gives precipitates with lead acetate, mercuric chloride,

Practical application of tests for albumin (See Urine).

(12) GLOBULIN is obtained from blood serum, from milk, from chyle, from the aqueous and vitreous humours of the eye, and from connective tissue.[1] In union with hæmatin it forms the colouring matter of the blood, "hæmoglobin." (See Blood.)

Preparation.—Rub up the fresh lenses of some bullocks' eyes with fine sand. Agitate with distilled water and filter. Collect the filtrate in a tall measuring-glass, and pass a current of carbonic acid gas through it, the globulin will be deposited. Allow the precipitate to settle, decant off supernatant fluid, and collect deposit on a watch-glass.

Chemical and physical characters—

Test 1. *It is insoluble in water.*—Place a small portion of the precipitate on a glass slide, add by means of a stirring-rod a few drops of distilled water; it is not dissolved.

Test 2. *It is soluble in dilute saline solution.*—Make 1 per cent. solution of sodium chloride. Add a few drops of this solution to the precipitate on the glass slide; it is dissolved.

Test 3. *It is precipitated by carbonic acid gas.*—This was shown in its preparation.

Test 4. *It becomes opalescent when heated to* 73° *C. and coagulates at* 93° *C.*—Try the experiment in the manner described for coagulation of albumin. (§ 11, Test 3.)

Para-globulin.—The researches of Hoppe-Seyler and C. Schmidt have shown, that the globulin obtained from blood serum differs from that of the crystalline lens in not being precipitated from its solutions by heat or alcohol, and also by the property it possesses

potas ium, ferrocyanide, and tannic acid; but it is not precipitated by magnesium sulphate.

[1] Globulin in its coagulated form is insoluble in water, but if the water be saturated with oxygen it is dissolved; it is also soluble in neutral saline solutions.

of coagulating certain liquids, as the pericardial, peritoneal, and hydrocele fluids, which do not coagulate spontaneously themselves. This modification of globulin has been called para-globulin, and also *fibrino-plastic* substance, from the power it has of forming with the above-named fluids, fibrin. It is readily obtained by passing a stream of carbonic acid gas through perfectly fresh blood serum, diluted with ten times its bulk of water; the precipitate is collected on a filter and thoroughly washed with water. If the fluid of a hydrocele be treated in the same manner, only the solution must be more dilute and the carbonic acid passed for a longer period, a coarse granular viscous precipitate, very much resembling para-globulin or *fibrino-plastic* substance, is thrown down. This substance has been called *fibrinogen*, from the fact that it requires to be mixed with fibrino-plastic substance to form fibrin.

(13) Myosin. This substance separates and coagulates from muscle soon after death; and it is this coagulation that most chiefly induces that condition of muscular rigidity known as "rigor mortis," which after lasting a variable period gradually passes off, as the myosin becomes decomposed.

Preparation.—The muscular tissue of an animal just killed is finely minced, and washed on a filter with a strong current of distilled water, till the washings give no precipitate with mercuric chloride to remove the soluble albumin, nor an acid reaction with test-paper. The residue on the filter is then treated with a 10 per cent. solution of sodium choride, and the filtrate allowed to drop into a beaker containing distilled water, when a

precipitate occurs. This must be allowed to settle, and then be collected on a watch-glass.

Chemical and physical properties.—Myosin thus obtained forms a gelatinous mass of greyish colour.

Test 1. *Insoluble in water.*

Test 2. *Soluble in dilute solution of sodium chloride, from which it is precipitated by the addition of sodium chloride in bulk.*—Dissolve some of the precipitate in half a test-tube full of solution of sodium chloride (10 per cent.); when a viscid-looking solution is formed add a pinch of dry sodium chloride; the myosin will be precipitated.

Test 3. *The saline solution of myosin becomes opalescent at* 55° *C. and is precipitated at* 60° *C.*—Try the experiment in manner described for coagulation of albumin (§ 11, Test 3).

(14) FIBRIN in the body is always in a fluid condition, and is found in the blood, chyle, and lymph. After removal from the body it undergoes spontaneous coagulation, and forms a firm laminated clot. The formation of fibrin, as we have stated before, is due to the action of fibrino-plastic substance (paraglobulin) on fibrinogen; if these two substances be mixed together in a liquid condition they combine and separate from the fluid as a jelly-like coagulum. Several circumstances may prevent or retard this coagulation; viz., extreme cold, the presence of carbonic acid, free acids—as acetic, phosphoric or lactic,—also alkalies and alkaline carbonates. The introduction of foreign bodies into the fluid hastens the coagulation.

Preparation.—Beat up some freshly-drawn blood, or better still, blood flowing from an animal, with a bundle of fine twigs; when all the fibrin is withdrawn from the blood, separate the fibrin from the twigs, place it in a muslin bag, tie the bag securely to a water-tap and allow

the water to run through for some hours, from time to time removing the bag and rinsing the fibrin ; continue this till the fibrin becomes free from colouring matter.

Chemical and physical properties.—Fibrin removed from the body is a soft elastic substance of filamentous texture, which under the microscope exhibits wavy, parallel fibres of granular appearance.

Test 1. *Insoluble in water.*—Place a small piece of fibrin on a glass slide and rub it with distilled water by means of a stirring-rod ; it is not dissolved.

Test 2. *Insoluble in dilute saline solutions.*—Place a small piece of fibrin on a glass slide, and rub it well with a 10 per cent. solution of sodium chloride ; it is not dissolved.[1] (If the process is continued a long time a portion of fibrin may become dissolved.)

Test 3. *Fibrin decomposes hydrogen peroxide.*—Place a few drops of hydrogen peroxide on a glass slide and add a fragment of fibrin ; brisk effervescence occurs.

II. Modified Albumins.

*(15) Lardacein. This substance is found deposited in the minute arteries of the liver, kidneys, spleen, intestines and brain, in certain diseases associated with long-continued suppuration, cachexia, &c. From the fact of its giving a blue coloration with iodine and sulphuric acid it was first regarded as a starch and received the name of "amyloid substance." It is however a nitrogenous body containing 15 per cent. of nitrogen, and has no affinity with any kind of starch. It is regarded as a modification of fibrin.

[1] The student should bear in mind the respective behaviour of globulin, myosin, and fibrin, in saline solutions. Globulin is readily soluble in a 1 per cent. solution ; myosin forms a viscid solution with a 10 per cent. solution, whilst fibrin is practically insoluble in a solution of any strength.

* *Preparation.*—Cut up the tissue, from which you wish to obtain it, into small fragments, and wash repeatedly with water and boiling alcohol till the mass is completely decolorized. Then digest the mass with artificial gastric juice in excess for some hours at a temperature of 40° C. Filter; the deposit in the filter will consist of lardacein mixed with small quantities of mucin and elastic tissue.

Chemical and physical properties—
Test 1. *Insoluble in water.*
Test 2. *Insoluble in dilute saline solutions.*
Test 3. *It is not digested by artificial gastric juice.*
Test 4. *It gives a red coloration when touched with an aqueous solution of iodine.*[1]
Test 5. *It gives a rosy red with anilin violet* (*methyl anilin*).

(16) CASEIN. (syn. *Alkali albumin*). All the albumins treated with dilute alkalis are converted into alkali albumin. The substance thus artificially obtained agrees in many respects with *Casein*, the albuminoid constituent of milk. It is also found in the serum of the blood. It can be obtained from muscle plasma and from the placenta. Casein also forms a large proportion of the albuminous matters of the nerve centres.

Preparation. *1. *From milk.*—Fresh milk is precipitated by an excess of magnesium sulphate, the precipitate washed with a saturated solution of magnesium sulphate, and redissolved in water. The aqueous solution is filtered, and the filtrate precipitated with dilute acetic acid; the precipitate is separated by filtration, and washed with ether to remove the fatty matters, and dried.

*2. *From blood serum.*—A current of carbonic acid gas is passed through fresh blood serum to remove the

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[1] This reaction is not so characteristic as has been supposed. Dried fibrin, syntonin, or acid albumin, and casein or alkali albumin, taking the same stain.

globulin; dilute acetic acid is then added, which precipitates the casein.

3. *Artificial.*—Place a small quantity of solution of egg albumin in a beaker, add an equal quantity of liquor potassæ; heat gently for a few minutes and set aside to cool.

Chemical and physical properties.—The reaction of casein[1] can best be studied with the artificially-prepared solution.

Test 1. *It is not coagulated by heat.*

Test 2. *Neutralized with acetic acid, casein is precipitated and is not redissolved on adding excess of acid.*

Test 3. *It is precipitated from its solutions by the addition of magnesium sulphate in bulk.*

Test 4. *Solutions of casein are not precipitated by acetic acid till after the point of neutralization is passed, if sodium or potassium phosphate be also present in solution.* —To a solution of casein add an equal quantity of solution of sodium phosphate, place a piece of blue litmus-paper in the solution. Add drop by drop acetic acid; notice the precipitate will not occur till some time after the litmus-paper has turned red.

(17) SYNTONIN (syn. *Acid albumin*). All the albumins treated with dilute acids are converted into acid albumin. The substance thus artificially obtained resembles closely the albuminoid obtained by treating muscle with very dilute hydrochloric acid, after all the soluble albumins had been removed by washing.

Preparation. *1. *From muscle.*—Mince muscular fibre very small, and place it on a filter; wash with a stream of cold water till the washings give no precipitate with

[1] According to Hoppe-Seyler, casein or natural alkali albumin differs from artificial alkali albumin in two respects. 1. Casein when treated with caustic potash yields potassium sulphide. 2. Digested with artificial gastric juice, casein forms a peptone containing phosphorus. Now artificial alkali albumin does not yield potassium sulphide with caustic potash, and its peptone contains no phosphorus.

mercuric chloride. Treat the residue on the filter with dilute hydrochloric acid (0·2 per cent.), and set aside for twenty-four hours; neutralize with sodic carbonate, and filter off the resulting precipitate, which is to be well washed with cold water.

2. *Artificially.*—Place a small quantity of solution of egg albumin in a beaker; add an equal quantity of dilute hydrochloric acid (made by diluting 6·25 cc of strong hydrochloric acid with 1 litre of water). Heat the mixture *very gently at first*,[1] gradually raising to boiling point; then set aside to cool.

Chemical and physical properties.—The reaction of syntonin can best be studied with the artificially-prepared solution.

Test 1. *It is not coagulated by heat.*

Test 2. *Neutralized with liquor potassæ it is precipitated. This precipitate is soluble with excess of liquor potassæ.*

Test 3. *Solutions of syntonin are precipitated by neutralization independently of the presence of sodium or potassium phosphate.* Proceed as with Casein (§ 16, Test 4). —This distinguishes acid from alkali albumin (§ 16, Test 3).

(18) Coagulated Albumin. When moist, it is a white opaque substance, which becomes yellow and brittle when dried. It is insoluble in water and dilute acids and alkalis; but soluble in strong acids and alkalis, which change it into acid and alkali albumin respectively.

(19) Peptones. These bodies are formed by the action of the gastric juice, or by weak acid solutions of pepsin, or by the alkaline pancreatic juice, on albuminous substances, at a temperature from 40°—50° C. The consideration of these bodies may be conveniently deferred till the demonstration on Digestion.

[1] The conversion of albumin into acid albumin does not take place immediately, therefore if too strong a heat were applied at first, coagulation would result.

III. Gelatins.

These principles are distinguished from the albumins by not being precipitated by potassium ferrocyanide with acetic acid, and in containing a smaller proportion of carbon and a larger quantity of nitrogen in their composition.

(20) Mucin is obtained from mucous fluids, such as bile and saliva. From the connective tissue of the embryo, from the submaxillary gland, and from tissues which have undergone mucoid and colloid degeneration.

Preparation. *1. *From bile.*—By precipitating with alcohol, washing precipitate with distilled water, redissolving in lime-water, filtering through animal charcoal, reprecipitating with acetic acid.

*2. *From the submaxillary gland.*—The submaxillary gland of an ox is rubbed down with pounded glass, and the mass placed in water for a night, then filtering and again treating the residue with water. The filtrate is precipitated by acetic acid, and the precipitate washed with water, acetic acid, warm alcohol, and then dried.

Chemical and physical properties—

Test 1. *Gives a stringy precipitate with alcohol.*—Place a few drops of saliva on a glass slide, pass a stirring-rod moistened with alcohol across, a white stringy precipitate of mucin will form. Try the same experiment with bile.

Test 2. *Gives a stringy precipitate with acetic acid.*—Proceed as above, only moisten the stirring-rod with acetic acid.

Test 3. *Soluble in lime-water.*—Add lime-water to the precipitate on the glass slide, then dissolve.

(21) Gelatin is obtained from bones, tendons, and areolar tissue, by long boiling in water. It is some-

times found in the blood of leucocythæmic patients, and in the juice of certain carcinomatous tumours.

Preparation. From bones.—Bones that have been thoroughly cleaned and dried are digested with dilute hydrochloric acid (1-20) till all the earthy matter is dissolved; the residue, which is called ossein, is then boiled for many hours, and dried at a temperature of 100°.

Chemical and physical properties.—Dry and pure gelatin is an amorphous transparent substance, hard and brittle, with no taste, or, if any, feebly sweet. Insoluble in ether and alcohol. In cold water gelatin swells up without dissolving. *Warm* water dissolves it, and the solution on cooling gelatinizes, even if the solution contains only 1 per cent. of gelatin; prolonged boiling destroys this property of gelatinizing.

Test 1. *Its solutions are precipitated by mercuric chloride and tannic acid.*

Test 2. *It is not precipitated by either acetic acid or alum* (*distinguishes it from chondrin*).

(22) CHONDRIN is obtained from cartilaginous tissue. Young bones prior to ossification, and adults' bones in certain diseased conditions, yield considerable quantities.

Preparation.—The costal cartilages of the calf are cut in thin slices, boiled for twenty-four hours, and the solution evaporated to a gelatinous consistence; the fatty matters are removed by digestion with boiling ether, and dried at a temperature of 100°.

Chemical and physical properties.—Chondrin is a diaphanous, horny substance, insoluble in alcohol and ether; on the addition of cold water it swells up (to about 12 times its original bulk), but is not dissolved; it dissolves freely in boiling water and forms a jelly on cooling.

Test 1. *Gives only a slight precipitate with tannic acid.*

Test 2. *Gives a precipitate with acetic acid.*

Test 3. *Gives a precipitate of alum soluble in excess.*

(23) *ELASTICIN. This substance is the special principle of yellow elastic tissue, and is consequently obtained from those textures in which this tissue is most abundant; as the yellow elastic ligaments of the vertebræ, the ligamentum nuchæ, the middle coat of arteries and veins, the areolar tissue, and the lower vocal cords.

**Preparation.*—The ligamentum nuchæ, or the middle coat of the arteries or veins, are boiled with alcohol and ether to remove the fatty matter; then treated with water at a temperature of 100° for twenty-four hours; and afterwards for one hour with water at 120°, this removes the other gelatinous principles; the residue is then boiled with strong acetic acid, and washed with water; again boiled with strong soda ley and treated with strong acetic acid and washed with water; finally the residue is digested with hydrochloric acid, washed with water, and dried.

Chemical and physical properties.—Elasticin forms a yellow, fibrous, brittle mass, soluble only in strong caustic alkalis; it is insoluble in water below the temperature of 120°. Its hot solution does not gelatinize on cooling.

Test 1. *Precipitates with tannic acid.*

Test 2. *No precipitate with acetic acid.*

Requisites for Demonstration III.

MATERIALS.—White of egg. Bullocks' eyes. Fresh fibrin or dried fibrin. Gelatin. Chondrin.

REAGENTS.—Distilled water. Alcohol. Ether. Nitric acid. Hydrochloric acid. Acetic acid. Tartaric acid. Tannic acid. Solutions of Ammonia. Sodium chloride. Sodium sulphate. Sodium carbonate. Sodium phosphate. Potassium hydrate. Potassium phosphate. Potassium ferrocyanide. Mercuric nitrate. Mercuric chloride. Cupric sulphate. Iodine. Methyl anilin.

APPARATUS.—Thermometer. Water bath. Carbonic acid gas generator. Fine sand. Filtering paper. Beaken. Test-tubes. Red and blue litmus-paper.

PART II.

DEMONSTRATION IV.

PRODUCTS OF DECOMPOSITION.

THE highly complex organic substances we have been considering, entering into the composition of the animal tissues and fluids, are decomposed in the body by the action of oxygen introduced into the economy by the process of respiration, into less complex bodies.

These products of decomposition are divided into two distinct groups, viz. : 1, the non-nitrogenous organic acids derived directly from the oxidation of the *saccharine* and *oleaginous* principles, and indirectly from the albuminous; and, 2, the nitrogenous bases which are obtained, together with the non-nitrogenous organic acids, from the oxidation of albuminoid principles. This oxidation is seldom, if ever, accomplished at one stage. *Intermediate* compounds of simpler constitution are commonly produced, but *ultimately* the final oxidation is reached, and thus the greater part of the elements of the food are

removed from the system in the forms of carbonic acid, water and urea.

I. CHIEF PRODUCTS OF THE DECOMPOSITION OF THE SACCHARINE AND OLEAGINOUS PRINCIPLES.

Intermediate { Lactic acid. / Oxalic acid.

Ultimate { Carbonic acid. / Water.

II. CHIEF PRODUCTS OF THE DECOMPOSITION OF THE ALBUMINOUS PRINCIPLES.

Non-nitrogenous bodies.

Intermediate { Lactic acid. / Oxalic acid.

Ultimate { Carbonic acid. / Water.

Nitrogenous bodies.

Intermediate { Xanthin. / Cystin. / Kreatin. / Leucin. / Tyrosin. / Hippuric acid. / Uric acid.

Ultimate—Urea.

In the above tables therefore we see that the Saccharine and Oleaginous principles break up into

a single series of non-nitrogenous fatty acids, *the lowest term of which is Carbonic acid.* The Albuminous principles by their decomposition furnish a double series; one of which is identical with the products of decomposition furnished by the saccharine and fatty bodies, whilst the other consists of certain nitrogenous bodies *the lowest term of which is urea,* the ammoniated form of carbonic acid.

I. Non-Nitrogenous Acids.

(24) Lactic Acid in a free state is found in muscle plasma, and gives to that fluid its acid reaction; the quantity present is increased by muscular contraction. Associated with hydrochloric acid it is almost invariably met with in the gastric juice. Unassociated with hydrochloric acid it is found in the large intestine, the product of fermentative changes. It is never obtained from healthy blood, since in that fluid its salts are speedily decomposed and converted into alkaline carbonates. In diseases, however, in which the oxidating power is diminished, as in some forms of dyspepsia, pyrexia, and pulmonary affections, lactic acid may be found in the blood and urine; its presence however in these fluids is generally due to fermentation. When present in the urine it is generally associated with an excess of calcium oxalate. In rachitic children the urine sometimes contains lactic acid, associated with an abundance of calcium phosphate; hence it has been suggested that an excess of lactic acid in the blood holds the calcareous salts in solution, and consequently they pass out of the system

in the urine instead of being deposited and forming bone.

It plays an important part in the performance of the functions of the body; 1, by its power of holding calcareous salts in solution it prevents the deposition of bony matter which would otherwise accumulate in all the tissues; 2, by the rapid combustion of its salts into alkaline carbonates it furnishes a supply of heat to the economy; and 3, by its presence in the intestinal canal during digestion it promotes the absorption of food into the blood-vessels and lacteals.

Chemical and physical properties.—Lactic acid is a colourless, syrupy fluid of sharp acid taste: sp. gr. 1·21. Soluble in water, alcohol and ether. It is formed in the body in three isomeric modifications. 1. Ordinary lactic acid (*ethylidene lactic acid*). This is the form usually met with in the stomach and intestines. 2. *Ethylene lactic acid* is found in the watery extract of muscles. 3. *Sarcolactic acid* exists, as its name implies, in muscular tissue in conjunction with the preceding form. These modifications can be distinguished from each other by the relative solubility of their respective zinc and calcium salts both in water and alcohol. The salts of ordinary lactic acid are the least soluble, the salts of sarcolactic acid being intermediate in degree of solubility.

* *To obtain lactic acid from an organic mixture.*—The fluid is gently evaporated, at a temperature a little below 100° C., to one-fifth its bulk, and then filtered; to the filtrate baryta is added and the precipitate removed by filtration; to the filtrate add a few drops of strong sulphuric acid and the mixture gently distilled; the residue left after distillation is then to be shaken with alcohol and allowed to digest. After standing some days it is filtered and the filtrate mixed with milk of lime and evaporated to dryness; the residue is dissolved in water and a stream of carbonic acid gas passed through the solution, which is to be heated to 100° C. When the solution is cold the precipitate is removed by filtration;

the filtrate evaporated to dryness, the residue dissolved in rectified alcohol, and the alcoholic solution concentrated and set aside; in a few days characteristic crystals of calcium lactate will be deposited.

(25) OXALIC ACID represents an intermediate stage in the oxidation of the more complex organic substances into carbonic acid and water. In health the oxalates are never met with in any of the fluids and secretions of the body, as they at once undergo oxidation, and are converted into carbonic acid and water; but if the process of oxidation be impeded then oxalates will be met with in the urine. We may therefore expect to find oxalates in urine in all cases of debility where the oxidizing power is diminished, as in dyspepsia, or in the convalescence after severe fevers, or in chronic diseases of the respiratory organs, as in chronic bronchitis, and emphysema. Oxalates are also formed in the urine after drinking carbonated beverages, as champagne, seltzer water, &c., and after eating certain fruits and vegetables, as rhubarb, sorrel, &c., which contain them in large quantities. With solutions of lime it forms the normal calcium oxalate CaC_2O_4, a salt of great interest to the pathological chemist. (See calcium oxalate, Urine).

(26) SUCCINIC ACID. This acid has been obtained from certain morbid exudations, as the contents of hydatid cysts, hydrocele, &c., by evaporating the fluids to the consistence of syrup, and adding hydrochloric acid and thoroughly exhausting the acid solution with ether; the etherial solution, on distillation, yields crystals of succinic acid.

Chemical and physical properties.—The crystals form large, rhombic, colourless tablets soluble in alcohol and cold water ; they are not decomposed by a high temperature, and can therefore be removed from organic mixtures by destructive distillation. Solutions containing succinic acid give a brown precipitate with ferric chloride, and white with barium chloride. The crystals when burnt in air give rise to intensely irritating and suffocating fumes.

II. Nitrogenous Bases.

*(27) Xanthin. This substance can be obtained from many of the tissues and secretions of the human body, as the liver, spleen, thymus gland, muscle, and the blood. It is not however found in normal urine, as in a healthy condition it undergoes immediate oxidation in the system and is converted into other products. Xanthin is a constituent of certain rare urinary calculi, and Dr. Bence Jones has recorded an interesting case of xanthin gravel occurring in a lad aged nine and a half years. The xanthin calculus removed by Langenbeck was also from a boy. Dr. Bence Jones considers that the xanthin diathesis will be found generally to occur in youth, as it is in the early period of life the greatest chemical variations of the body are to be expected, and the imperfect oxidation of xanthin into uric acid most likely to occur.

Preparation from urine.—Add baryta water to the urine supposed to contain xanthin, till a precipitate is no longer thrown down ; filter, and evaporate the filtrate to a syrup, and allow it to crystallize. The mother-liquor, after the removal of the crystals, is boiled with cupric acetate, and the precipitate thus formed is removed by filtration, washed, and dissolved in warm nitric acid. This acid

solution is precipitated by silver nitrate, and the resulting precipitate washed and crystallized from hot dilute nitric acid, and the crystals washed with ammoniacal silver solution, and suspended in water. The aqueous solution is to be decomposed with sulphydric acid, filtered, and the filtrate evaporated ; the residue yields xanthin.

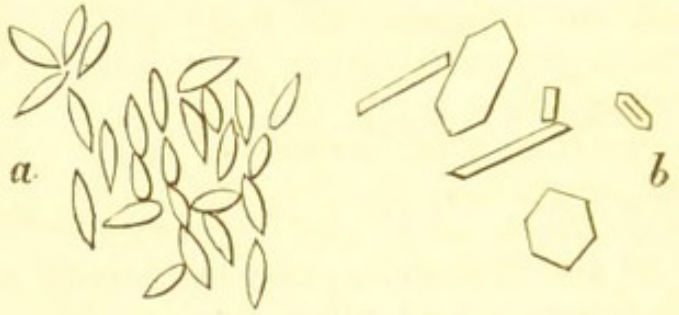

FIG. 2.—Xanthin.

Chemical Properties.—Xanthin forms white scales, somewhat resembling bees-wax in appearance. Deposited spontaneously from urine it occurs in lemon-shaped plates, (*a*) those dissolved by hydrochloric acid and the solution evaporated, yield prismatic and hexagonal crystals. (*b*) Xanthin is insoluble in water, alcohol, and ether ; soluble in alkaline solutions. *Evaporated with nitric acid on platinum foil and the residue moistened with liquor potassæ, it yields a dark purple colour.*

* (28) CYSTIN. According to Dr. Bence Jones cystin is constantly being separated in the healthy organism, immediately undergoing transformation into sulphuric acid, carbonic acid, and urea. Whenever this chemical transformation is arrested cystin appears in the urine. Calculi composed of cystin are found occasionally in the kidney and urinary bladder, they are, however, rare. They are usually soft and compressible, have a waxy appearance externally, and are of a greenish yellow colour.

Preparation.—The urine supposed to contain cystin is acidulated with acetic acid and allowed to stand till a

deposit is formed. This deposit is dried, redissolved in ammonia, and the ammoniacal solution evaporated; cystin, if present, will be recognised in the residue by the hexagonal form of its crystals.

Chemical and physical properties.—The crystals form hexagonal laminæ, which overlay each other, forming little groups; they have a pale lemon colour, which, on exposure to light and air, acquires a greenish tinge. They are *soluble in strong nitric acid, and the acid solution evaporated with ammonia does not give the murexide reaction (distinguishing them from uric acid crystals.)* Caustic alkalis also dissolve cystin, the alkaline carbonates precipitate it from its acid solutions, and acetic acid from its alkaline solution.

FIG. 3.—Cystin.

Boiled in a solution of caustic potash with a little lead acetate, a black precipitate of lead sulphide is thrown down, which is due to the presence of sulphur contained in the cystin.

*(29) LEUCIN. This substance, associated with tyrosin, may be obtained from all glandular organs and their secretions; it is especially abundant in the lung and liver tissue. It is never found in normal urine, its presence in that fluid always denoting serious disease; thus, it has been met with in the urine in severe cases of jaundice, in acute yellow atrophy of the liver, in cirrhosis of that organ, and in cases of smallpox and typhus; in these diseases the quantity is also increased in the other organs of the body.

FIG. 4.—Leucin in oily discs.

Preparation.—From urine, if present, evaporate about five ounces of that fluid to a thin syrup, and when cold, leucin in the shape of oily circular-looking discs will be deposited. Dissolve in boiling alcohol.

Chemical and physical properties.—Leucin is deposited from its cold alcoholic solution in white shining plates greasy to the touch, lighter than water, and much resembling cholesterin in appearance, *it is distinguished from that substance by its insolubility in ether.* It is slightly soluble in cold water, and very soluble in boiling water, very insoluble in ether.

*(30) Tyrosin. Associated with leucin it has been obtained from all the glandular organs and secretions of the body. So constant is the association of these two bodies, that they are considered to be products of the metamorphoses of the same kind of tissue. They are never found in normal urine, their presence in that secretion always denoting serious disease.

Preparation. From urine.—Precipitate the colouring and extractive matters with basic lead acetate, and filter; decompose the filtrate with sulphydric acid, and filter; the clear filtrate is to be concentrated, and, on cooling, crystals of tyrosin will be deposited.

Chemical and physical properties.—The crystals are long prismatic needles, which cluster together to form

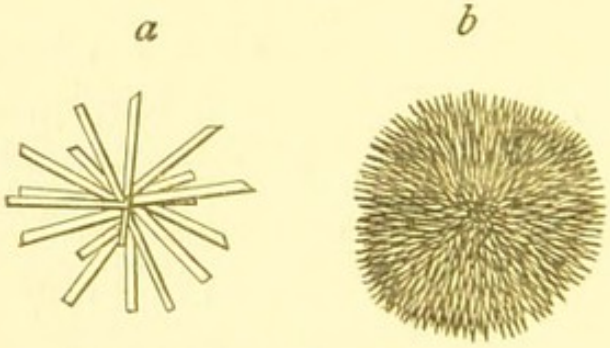

Fig. 5.—Tyrosin.
a. Stellate crystal. *b*. Spherical ball.

stellate groups; sometimes, when obtained from urine, these groups are so closely aggregated together as to form balls of spiculated needles. The crystals are sparingly soluble in cold water and alcohol; soluble in acid and alkaline solutions; insoluble in ether.

Warmed with a few drops of sulphuric acid, a solution of tyrosin gives, after neutralization with barium carbonate, a violet reaction with ferric chloride. Treated with strong nitric acid, a yellow substance, nitrate of nitrotyrosin, is formed, which with hydrochloric acid gives a red, and with ammonia a brown coloration. A solution of tyrosin heated with a mixture of mercuric and mercurous nitrate, gives a red precipitate.

*(31) KREATIN. This substance is one of the primary products of muscle decomposition; and is always found in the juice of muscular tissue. In the blood it is decomposed either into urea or kreatinin. Large quantities of kreatin are obtained from meat extract, but as keatin is an effete product, it has little nutritive value, the amount of force liberated in its conversion into urea and kreatinin being inconsiderable. It is a question whether kreatin is a constituent of normal urine; whenever, however, it is not burnt off in the system into urea and kreatinin, it is found with the latter substance in the urine.

Preparation.—Is most readily obtained from meat extract. Dissolve one ounce of Liebig's extract of meat in one pint of water, add a solution of basic lead acetate till a precipitate is no longer formed. Filter; decompose filtrate with sulphydric acid; again filter. Evaporate filtrate to a syrupy consistence, set aside for a few days, when crystals of kreatin will be deposited.

Chemical and physical properties.—The crystals are prismatic, colourless and brilliant, having a bitter taste and giving a neutral reaction with test-paper; tolerably soluble in cold water, very soluble in boiling water. Kreatin by the action of hydrochloric acid is converted into kreatinin. Boiled with baryta water it is decomposed into urea.

*(32) KREATININ. This powerful base is constantly

present in human urine ; according to Neubauer the quantity passed into the urine in twenty-four hours averages 0·6 to 1·3 grm. It is derived from the decomposition of kreatin in the blood ; in no case has it been obtained as a primary product of decomposition from any of the tissues. It has been shown by recent experiments that it does not occur in muscular tissue either at rest or when tetanized.

Preparation. From urine.—To 250 c.c. of the twenty-four hours' urine, add milk of lime, till the urine has an alkaline reaction ; calcium chloride is then added till a precipitate ceases to be formed ; filter and wash. Evaporate the filtrate and washings to a syrupy consistence and add 50 c.c. of alcohol ; set the mixture aside in a cool place and filter off any precipitate that may form. The clear filtrate is then evaporated to 50 c.c., and when quite cold ½ c.c. of the alcoholic standard solution of zinc chloride (see Appendix) is added ; the mixture is set aside in a dark cool place, and after twenty-four hours, crystals of kreatinin zinc chloride will form. The crystals are collected on a weighed filter, washed with alcohol, till the washings are colourless, and the filter dried with the crystals between two watch-glasses ; and weighed. 100 parts of kreatinin zinc chloride represent 62·4 parts of kreatinin. To obtain kreatinin from the zinc compound, the latter must be boiled for some hours with an excess of hydrated lead oxide ; the mixture is then filtered through animal charcoal, and evaporated to dryness, the residue treated with boiling alcohol, and the alcoholic solution concentrated ; on cooling, the alcoholic solution will deposit crystals of kreatinin.

Chemical and physical properties.—The crystals form oblique rhombic prisms, which are soluble in boiling water, soluble in 12 parts of cold water, and in 100 parts of absolute alcohol. It is an extremely powerful base, gives an alkaline reaction with test-paper ; and forms well-defined basic double salts, as with zinc chloride, mercuric chloride and silver nitrate.

(33) HIPPURIC ACID. This substance is a normal constituent of human urine, the quantity passed in the twenty-four hours under ordinary circumstances varying from 0·8 to 1 grm.

The excretion is greatly augmented by a vegetable diet, and especially by such vegetable substances as benzoic acid, cranberries, blackberries, and plums. Consequently we are not surprised to find a considerable quantity in the urine of all herbivorous animals; thus cow's urine contains 1 per cent., and horse's urine 0·38. In these animals hippuric acid often undergoes oxidation in the system and is converted into benzoic acid, which appears in the urine; thus horses at rest pass urine free from benzoic acid and containing the standard quantity of hippuric acid, but when put to hard work the hippuric acid diminishes and benzoic acid appears.

Kühne has observed that benzoic acid given to patients suffering from disease of the liver passes unchanged into the urine instead of being converted into hippuric acid, which would have been the case under ordinary circumstances. From this fact he has assumed that hippuric acid is derived from the vegetable aromatic constituents of our food and the place of transformation is the liver.

The excretion of hippuric acid is increased in all febrile affections, also in diabetes.

Preparation. **From urine.*—Evaporate 1,000 c.c. of urine to near dryness, triturate the residue with clean sand and add 60 c.c. of hydrochloric acid; finally, extract with alcohol. The acid alcoholic solution is neutralized with soda ley, and evaporated to a syrupy consistence

with a small quantity of oxalic acid, the residue dried in a water-bath and treated with a large quantity of ether containing 20 per cent. of alcohol. When the residue is thoroughly exhausted, the alcoholic etherial solution is evaporated and the crystalline residue treated with a solution of milk of lime, and the resulting precipitate removed by filtration. The filtrate is concentrated and hydrochloric acid added; after standing some hours hippuric acid will crystallize out. The crystals are collected on a weighed filter, dried and weighed; the weight gives the quantity of hippuric acid in the amount of urine examined.

Chemical and physical characters.—The crystals are semi-transparent pointed, rhombic prisms; almost insoluble in cold water and acetic acid; soluble in alcohol. When deposited in urine they may be mistaken for crystals of triple phosphate or uric acid. *Their not dissolving in acetic acid proves that they are not triple phosphate; whilst their solubility in alcohol will show that they are not uric acid.* Hippuric acid is monobasic, and the hippurates all give buff-coloured precipitates with ferric salts.

(34) Uric Acid (*syn. Lithic Acid*) is always present in small quantities in human urine, it has also been found in the blood, the spleen, and liver. In birds, reptiles, and insects whose tissue metamorphosis is represented by this product rather than urea, the semi-solid excrement is almost entirely composed of ammonium urate.

Uric acid represents one of the intermediate products of the "retrograde metamorphosis" of the albuminous constituents of the body; and in all mammalia is itself decomposed in the blood into urea and carbonic acid.

The quantity of uric acid daily eliminated with the urine in the twenty-four hours varies considerably.

Parkes states the average to be about 0·5 grm. or about $7\frac{1}{2}$ grains. Uric acid is never found free in normal urine but always in combination with soda, potash and ammonia, forming soluble salts, the *urates* or *lithates*. Whenever free uric acid or a deposit of urates occur in the urine, some abnormal condition of the system is indicated. (See Urinary Deposits. Urine).

Preparation. From urine.—Place 200 c.c. of urine in a tall urine glass, add 20 c.c. of strong hydrochloric acid. Set aside in a dark cool place for 24 hours, at the end of that time crystals of uric acid will be deposited on the sides and bottom of the glass. Collect the crystals on a watch-glass, and wash with alcohol acidulated with a few drops of hydrochloric acid.

Chemical and physical properties.—The crystals of uric acid obtained from human urine present a great variety of forms; the most common are the smooth, transparent,

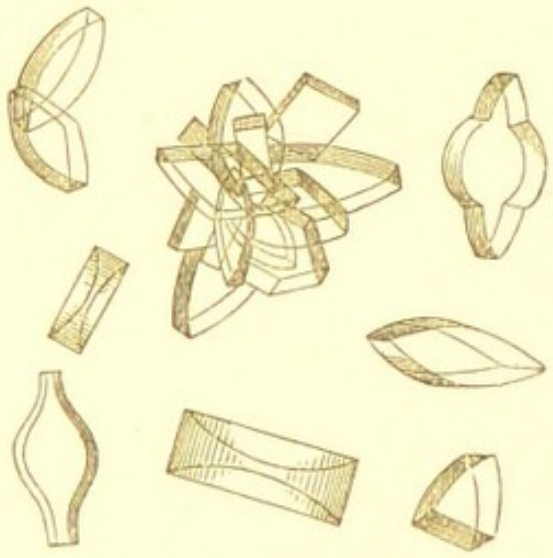

FIG. 6.—Uric Acid.

rhomboidal tables of variable size; mixed with these are diamond-shaped plates, hexagonal tables, rectangular, four-sided prisms, bundles of needle-shaped crystals, saw-like and dentated. If these mixed crystals are dissolved in

liquor potassæ, and recrystallized by the addition of hydrochloric acid, the characteristic rhomboidal tables become more evident.

Test 1. *Extremely insoluble in cold water.*—Place a crystal on glass slide, add water with stirring-rod, it will not dissolve.

Test 2. *Readily soluble in alkalies.*—Add a drop of liquor potassæ to crystal, it will dissolve.

Test 3. *Insoluble in acids.*—Add a drop of hydrochloric acid to alkaline solution (Test 2), the uric acid will be reprecipitated.

Test 4. *Gives a violet red coloration* (*murexide*) *when evaporated with nitric acid, and the residue touched with ammonia.*—Place a few crystals in a white porcelain dish, and add a drop or two of nitric acid with a stirring-rod; evaporate gently; when residue is dry touch with ammonia, a violet red colour will appear.

(35) Urea. This substance represents the ultimate product of the oxidation in the body of albumin and the albuminous tissues, derived from the further oxidation of those imperfectly oxidized substances xanthin, uric acid, kreatin, &c., which are the earliest products formed in the retrograde metamorphosis of albumin. The view most generally adopted at present regards urea as representing the metamorphosis of the whole of the nitrogenous elements of the food which have undergone conversion into tissue. Dr. Parkes has shown that if the body be kept perfectly quiescent, and varying quantities of nitrogenous food given, the excretion of urea is always in direct proportion to the nitrogen ingested. He has also shown, by a series of observations made on individuals, that all the nitrogen taken as food was recoverable from the fæces and urine, just the same, whether the body was at rest or at work.

The average quantity of urea which passes into the urine per diem may be stated at 30 to 40 grms. The quantity, however, is subject to variation, depending on the quantity and nature of the food, the age, sex, body weight, and health of the individual.

Dr. Parkes gives the quantity of urea excreted in 24 hours as $3\frac{1}{2}$ grains from every 1 lb. of body weight; consequently we may expect a larger elimination in heavy than in light persons. This relative proportion may hold in some cases, but after a certain weight is attained, is not at all to be depended on.

In chronic diseases, as phthisis (when hectic is absent), and especially in chronic albuminuria, the quantity is materially diminished. When the liver is much disorganised by disease, as in cancer, cirrhosis, and abscess of that organ, the quantity of urea formed is much decreased.

Preparation. From urine.—By evaporating the urine to a syrupy consistence, and treating it with nitric acid; sp. gr. 1·25. The urea nitrate thus formed is decomposed by a solution of barium carbonate, and the mixture evaporated; the residue is treated with boiling alcohol, and filtered · the filtered solution yields, on cooling, crystals of urea.

Chemical and physical properties.—Urea crystallizes in colourless four-sided prisms, neutral to test-paper.

Test 1. *Extremely soluble in cold water.*—Place a crystal on glass slide, touch with a drop of water; the crystal readily dissolves. The solution is neutral to test-paper.

2. *Touched with nitric acid, nitrate of urea is formed.*—Touch the solution of urea on the glass slide with a drop of nitric acid, a white shining precipitates, consisting of characteristic crystals, rhombic plates of nitrate of urea.

Test 3. *Touched with oxalic acid, oxalate of urea is formed.*—To a few drops of solution of urea add a drop or two of a concentrated solution of oxalic acid; a white precipitate of oxalate of urea will slowly form.

FIG. 7.—Nitrate of urea.

4. *Mercuric nitrate forms with urea an insoluble compound.*—Touch a drop of solution of urea with a drop of mercuric nitrate, a thick curdy precipitate will result.

(For methods of estimating urea quantitatively, see *Urine*.)

Appendix to Demonstration IV.

The products of decomposition which have not been considered in the foregoing demonstration may be enumerated briefly as follows. 1. *Cholic acid* is a non-nitrogenous acid found in bile, combined with glycocin and taurin, forming with these substances the bile acids (§ 46). When bile has been removed some time from the body the bile acids undergo decomposition and set free cholic acid. Cholic acid heated with acid at temperatures of 200° C. is converted into dyslysin (so named from its insolubility in water, acids, alkalies and alcohol). 2. *Glycocin* is a nitrogenous base derived from the decomposition of the gelatinous principles of the body; it does not exist in a free state in the economy, but conjugated with cholic acid it forms glyco-cholic acid (§ 46). It is also a residue of hippuric acid, from which it can be obtained by boiling a few crystals in a test-tube with strong hydrochloric acid; on cooling glycocin will crystallize out. 3. *Taurin* is a nitrogenous base, associated with cholic acid; it is a constant constituent of bile; forming tauro-cholic acid, § 46. It is also obtained from all glandular organs, especially the lungs, and from voluntary muscular fibre. 4. *Cholin* (syn. *Neurin*). This energetic nitrogenous base was obtained originally by Strecker in 1861 from pigs'

bile ; it is chiefly associated with lecithin. It is apparently one of the products of the metamorphosis of nervous tissue. 5. *Cerebrin*, *Lecithin*, and *Oleophosphoric acid* will be considered fully in the demonstration on nervous matter.

Requirements for Demonstration IV.

MATERIALS.—Hippuric acid. Uric acid. Urea.[1]

REAGENTS.—Alcohol. Acetic acid. Oxalic acid. Nitric acid. Hydrochloric acid. Solutions of ammonia. Potass hydrate. Ferric chloride. Mercuric nitrate.

APPARATUS.—Test-tubes. Glass slides. Stirring-rod. Litmus-paper. White porcelain dish.

[1] Small quantities of these substances can be obtained by the student for experiment from Mr. Martindale, 10, New Cavendish Street, W.

DEMONSTRATION V.

DIGESTION.

DIGESTION is the process by which the insoluble materials of the food are broken up and are rendered soluble by chemical changes effected by the digestive fluids, thus :—

Insoluble Material	Converted into soluble	By the agency of	Secretion.	Reaction of
Starch.	Grape sugar.	Ptyalin. Diastase.	Saliva. Pancreatic juice.	Neutral or alkaline. Alkaline.
Albumin.	Peptones.	Pepsin. Trypsin.	Gastric juice. Pancreatic juice.	Faintly acid. Neutral or alkaline.
Fat.	Emulsionized and saponified.	? Alkaline bases aided probably by an emulsive ferment.	Bile. Pancreatic juice.	Neutral or alkaline. Alkaline.

(36) SALIVA. In the mouth the food is thoroughly broken up, triturated, and mixed with the saliva. The

fluidity of the secretion separates the particles of the food and allows the digestive fluids to act more freely on them; and its viscidity greatly assists the act of deglutition. The conversion of the starchy matters of the food into glucose commences in the mouth, but the change is only partially effected at this stage of the digestive process.

The saliva is a secretion furnished by the parotid, submaxillary, and sublingual glands; from which it can be obtained by introducing canulas into their respective ducts. In the mouth it becomes mixed with the oral mucus. Thus mixed, it is a turbid, viscous fluid, with an alkaline reaction, specific gravity 1·018–1·025; it possesses the power of converting starch into glucose, which is due to *ptyalin*.

The fluid secreted by the submaxillary gland differs from that secreted by the parotid and sublingual glands, in containing mucin; and in man saliva from the submaxillary gland has more activity in converting starch into glucose than that furnished by the parotid and sublingual glands.

(37) PTYALIN, the substance which gives saliva the power of converting starch into grape sugar, is regarded as a *hydrolytic* ferment, as its action seems to depend on its power of splitting up a molecule of starch with the assumption of water, thus :—

$$\underset{\text{Starch.}}{3(C_6H_{10}O_5)} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Dextrin.}}{2C_6H_{10}O_5}.$$

Ptyalin exists in very small quantity, and has never been isolated in such a state that its nature can be satisfactorily demonstrated. Its action upon starch

is most powerful in dilute alkaline solutions, and at a temperature of 40° C. If strong acids or alkali are added, or the temperature is raised above 60° C., its action is destroyed.

A glycerine extract of ptyalin may be made by digesting a fresh salivary gland finely minced with glycerine, or by precipitating fresh saliva with normal phosphoric acid and lime-water ; filtering off precipitate and dissolving it in distilled water ; from the clear solution thus obtained the ptyalin is precipitated by alcohol ; the precipitate collected washed with distilled water and dissolved in glycerine.

The composition of saliva as obtained from the mouth is as follows: water, 99·4, solids, 0·6. The solids consist of (1) traces of ordinary albumin and globulin ; (2) mucin ; (3) ptyalin ; (4) salts, chiefly calcium carbonate, and usually traces of potassium sulphocyanate.

To demonstrate the presence of these, proceed as follows :—

ORDINARY ALBUMIN.—1. Evaporate a small quantity of saliva in a porcelain dish with a few drops of nitric acid ; touch the dried residue, by means of a stirring-rod, with ammonia ; the residue becomes orange-coloured (*Xantho proteic reaction*). 2. Place a small quantity of saliva on a glass slide, touch with a mixture of potassium ferrocyanide and acetic acid, a white precipitate is deposited. 3. Heat a small quantity, it becomes turbid (§ 11, Test 3). 4. Place a small quantity of saliva in a glass and touch with nitric acid, it is coagulated (§ 11, Test 4).

MUCIN.—Draw a stirring-rod moistened with acetic acid through some saliva placed on a glass slide, a stringy precipitate will follow. This precipitate will be redissolved on the addition of lime-water.

PTYALIN.—The presence of ptyalin is shown by the conversion of starch into glucose. Add a small quantity of saliva to half a test-tubeful of solution of

starch (1 part of starch to 25 water), and place it in water-bath at 40° C. for a few minutes, then apply Test 5, Glucose, § 3.

SALTS.—The chief is *calcium carbonate*, which, deposited from saliva, forms the tartar on the teeth, and is also the chief constituent of salivary calculi. Evaporate some saliva to dryness on a porcelain dish, scrape off the residue and place it on a glass slide, touch with hydrochloric acid; effervescence, denoting escape of carbonic acid gas, ensues. Touch the solution with ammonium oxalate, a white precipitate of calcium oxalate will form. *Potassium Sulphocyanide* (not always present in saliva). Evaporate some saliva on a porcelain dish, touch residue with dilute solution of ferric chloride; a deep red colour develops, which disappears on the addition of mercuric chloride.[1]

Various circumstances influence the rapidity with which saliva converts starch into sugar, thus :—

The action of saliva is most active on starch at a temperature of 40° *C. Cold, and temperatures above* 60° *C., also strong acids and alkalies, destroy its action.*—Take five test-tubes and place in each 1 c.c. of saliva and label them *a*, *b*, *c*, *d*, *e*. To test *a* add 5 c.c. of cold solution of starch and place in water-bath at temperature 40° C. Do the same to test-tube *b*, but place it in cold water. Boil the saliva in test-tube *c* over gas-flame, and then add 5 c.c. of starch solution. Add to test-tubes *d* and *e* a few drops of strong hydrochloric acid and liquor potassæ respectively, then add 5 c.c. of starch solution. After a short interval test for glucose (§ 3, Test 5). Test-tube *a* will yield a copious reddish yellow precipitate of reduced copper; test-tube *b* comparatively a slight reduction; whilst test-tubes *c*, *d*, and *e* will not give the characteristic reaction for sugar at all.

(38) GASTRIC JUICE is an acid, glairy, amber-coloured fluid secreted by the peptic gastric glands. Obtained from a gastric fistula during an early stage

[1] This distinguishes it from meconic acid.

of digestion, it has a specific gravity of about 1·010, containing about 2 per cent. of solid matter. The active property of gastric juice is due to a ferment *pepsin* in conjunction with *free acid.*

(39) PEPSIN is generally prepared by separating the mucous membrane from the muscular tissue of the stomach of a recently-killed animal, rejecting the pyloric extremity, and macerating it in dilute phosphoric acid till it is completely dissolved. The acid solution is then precipitated with lime-water, the precipitate removed by filtration, washed and redissolved in dilute hydrochloric acid, and the filtrate placed in a glass flask. To this acid solution, a saturated solution of cholesterin in 1 part of ether and 4 of alcohol, is added gradually by means of a long filter passing down to the bottom of the vessel, and the whole mixture well agitated. The cholesterin immediately separates and rises to the surface, bringing the pepsin with it; this mixture of cholesterin and pepsin is then separated by filtration, and the cholesterin removed by repeated agitation with ether.

For practical purposes of demonstration a glycerine solution of pig's stomach may be employed, and is thus prepared:—

* Separate the mucous membrane from the muscular tissue of a recently-killed animal, rejecting that part near the pylorus. Mince very fine and place in a glass beaker, and add just sufficient glycerine to cover the contents. After standing two or three days decant off the glycerine, leaving as much residue behind as possible. Add more glycerine to residue and again remove after a day or so; repeat this extraction three or four times. The glycerine

removed each time holds pepsin in solution, and this solution filtered through linen and kept in stoppered bottle is reserved for use.

The following reactions should be studied with the glycerine solution of pepsin.[1]

Exp. 1. *Pepsin by itself has no action on albuminous substances.*—Put a small quantity of finely mixed muscle in a test-tube and add some glycerine solution of pepsin. Place the test-tube in water-bath at temperature of 40° C. No change will occur (verify by Tests 1, 2, 3, § 44).

Exp. 2. *Pepsin in conjunction with dilute acid converts albuminous substances into peptones.*—Proceed as above, but add to the glycerine solution an equal quantity of dilute acid (0·2 per cent. of hydrochloric acid). Place in water-bath at temp. 40° C. After a while the muscle swells and becomes translucent, and apparently splits up, and finally is reduced to a fine granular debris. Filter, and examine filtrate for peptones by Tests 1, 2, 3, § 44.

Exp. 3. *Pepsin is precipitated by alcohol and by both neutral and basic lead acetate.*—Place three separate drops of glycerine solution of pepsin on a glass slide and touch with alcohol and with basic and neutral lead acetate, a precipitate occurs.

Exp. 4. *Pepsin is not precipitated by strong nitric acid, tannic acid, or mercuric chloride.*—Proceed as above.

(40) Free Acid. The acid concerned in gastric digestion is undoubtedly hydrochloric; though lactic and other organic acids may be also present, these are probably derived from fermentive changes occurring in the stomach. The acidity of gastric juice is however feeble, being equivalent to 0·15 to 0·2 per cent. of real hydrochloric acid.

To prepare 0·2 per cent. solution of real hydrochloric acid.—Take 6·2 c.c.'s of ordinary hydrochloric acid and place them in a glass flask graduated to hold one litre

[1] If the student cannot readily obtain a fresh pig's stomach, glycerine solution of prepared pepsin may be used.

(1000 c.c.'s); fill up to the mark on the neck of the flask with distilled water.

(41) The nature of gastric digestion may be conveniently studied with an artificial gastric juice made with the glycerine solution of pepsin and 0·2 per cent. solution of hydrochloric acid; in the following proportions, one-fourth glycerine extract, three-fourths acid solution.

Exp. 1. On Albumins. *Dissolves albuminous substances, converting them into peptones.* — Proceed as directed in § 39, Exp. 2.

Exp. 2. On Milk. *The casein of the milk is coagulated before being converted into peptone.*—Add some artificial gastric juice to milk, a curd is formed which gradually dissolves as the digestive action proceeds. (*a*) *This curdling is caused by some ferment which sets up lactic acid fermentation of the milk sugar and is not due to the acid of the juice.* To show this, neutralize the artificial gastric juice with 0·1 per cent. solution of sodium carbonate, the curdling takes place just the same. (*b*) *Heat destroys the power of the ferment to produce curdling.* Boil the artificial gastric juice before adding it, no curdling takes place.

Exp. 3. On Gelatin. *It dissolves gelatiferous tissues.* —Dissolve some gelatin in boiling water and place about a drachm of the hot solution in two test-tubes, *a*, *b*. When the contents have cooled down to about 40° C., add to test-tube *b* some artificial gastric juice and digest it for an hour in water-bath at 40° C. Set both aside to cool, it will be found that the solution in test-tube *a* has completely gelatinized, whilst that in test-tube *b* is still fluid or only imperfectly gelatinized.

Exp. 4. On Starch. *Gastric juice has no action on the starchy matters of the food*, i.e. *does not convert them into glucose.*—In a test-tube add to solution of starch an equal bulk of artificial gastric juice, digest in water-bath at 40° C. The reaction for glucose will not be obtained with Test 5, § 3.

Exp. 5. On Fats. *Gastric juice dissolves the albuminous envelopes of the fat cells, and the temperature* 40°

C., at which digestion is carried on, renders the contents fluid, but no chemical change occurs. — Place a small fragment of mutton suet in the bottom of a test-tube and half fill with artificial gastric juice. Digest in water-bath at 40° C. After a while the suet dissolves and oil globules float free on the surface of the mixture. Remove from water-bath and shake, a slight milkiness or emulsion, due to a minute subdivision of the oil globules, results. On standing, however, these globules slowly unite and form larger ones, and finally, a considerable portion of the oil will separate and float free on the surface of the mixture, showing that no permanent change has been effected.

(42) Circumstances influencing the action of gastric juice on albuminous substances :—

Exp. 1. *Gastric digestion proceeds best at temperature* 35° *to* 40°. *Boiling destroys all action.*— Place some finely minced muscle or boiled fibrin, about as much as will lie on a threepenny-piece, in three test-tubes, *a*, *b*, *c*. Fill each tube two-thirds full of artificial gastric juice. Place tube *a* in water-bath at temperature 40° C. ; tube *b* in water at temperature 60° C. ; tube *c* at the ordinary temperature. At the end of an hour compare the three tubes ; the change in *a* will be great, in *b* and *c* very slight. Mix contents of *b* and *c*, and divide into two portions. Boil one portion over gas-flame, and then place in water-bath at 40° C. ; no action will occur, however long the tube may remain in bath. Place the other portion, without boiling, to water-bath at 40° C., digestive action soon commences.

Exp. 2. *Neutralization, or the presence of too much acid, arrests the action.*—Prepare three test-tubes as above with minced muscle and artificial gastric juice. Neutralize *a* with 1 per cent. solution of sodium carbonate ; add to *b* a few drops of strong hydrochloric acid ; leave *c* and place the three tubes in water-bath at temperature 40° C., digestion alone will proceed in test-tube *c*. (N.B. If the mixture in test-tube *a* acidified by the addition of 0·2 per cent. solution of hydrochloric acid ; and if the contents of test-tube *b* be diluted with water till rendered faintly acid, digestion will proceed, showing the action has only been arrested, not destroyed, by neutralization, or by the presence of too much acid.)

Exp. 3. *The concentration of the products of digestion hinders digestion.*—Fill one-third of a medium-sized test-tube with finely minced muscle, or boiled fibrin, and add artificial gastric juice up to two-thirds ; place in water-bath at 40° C. Up to a certain point digestive action goes on ; then apparently stops. Now remove to larger test-tube and fill up with 0·2 per cent. solution of hydrochloric acid, and place again in water-bath 40° C. ; digestion is again resumed, and after a while again stops. Now remove to beaker, and keep on adding the dilute acid for a time, till the whole is dissolved. (N.B. This shows that the action of pepsin is continuous and that it is not destroyed.)

Exp. 4. *Minute subdivision by increasing the surface acted on by the gastric juice favours digestive action.*— Place an equal weight of finely minced and coarsely chopped muscle in test-tubes *a* and *b* respectively ; add to each an equal quantity of artificial gastric juice and digest at 40° C. The finely divided muscle will be digested hours before the more coarsely chopped.

(43) Peptones. If to a beaker one-third full of solution of albumin, an equal quantity of hydrochloric acid (0·4 per cent.[1]) be added and the beaker placed in the water-bath at temp. 40° C., after a time the whole, or nearly the whole, of the albumin will be found to be changed into acid albumin or syntonin (§ 17), and will be precipitated by neutralization of the solution with a 0·1 per cent. solution of sodium carbonate. When, however, a glycerine solution of pepsin is added to a similar mixture, it will be found that after an equal interval of time the precipitate obtained by neutralization with sodium carbonate is much less than is the case with the mixture of albumin and dilute acid only, and that another

[1] The acid should be made of this strength, as the solution of albumin reduces its strength to 0·2 per cent.

product remains in the solution, which can be shown to be peptone (§ 43, Tests A. 1, 2, 3, B. 1, 2, 3). The longer the mixture is exposed to the digestive action the less will be the quantity of syntonin precipitated on each neutralization with sodium carbonate, and the greater will be the amount of peptone found in the filtrate; till when the digestive action is completed the precipitate thrown down will be extremely slight, whilst the product in the filtrate will be considerable, showing that nearly all the albuminous element has been converted into peptone. As the precipitate obtained by neutralization with sodium carbonate differs in some respects from the acid albumin obtained by the action of dilute acid or albumin, the term *para-peptone* has been given it. Before the final conversion *into true* peptone several *intermediate*[1] products are formed to which different names have been assigned. Since the question with regard to the nature of these products, as will be seen by reference to the foot-note, is still in an unsettled state, it will be sufficient for the student to identify the presence of 1, *para*-peptone; 2, *intermediate* peptone; 3, *true* peptone.

[1] Meissner distinguishes these intermediate products by the names of *meta-peptone* and *A* and *B* peptones; the true peptone, the final product of gastric digestion, he calls *C* peptone. Kühne considers that both peptic and pancreatic digestion give rise, first to two products which he terms *anti-albuminose*, equivalent to *para-peptone*, and *hemi-albuminose*, equivalent to Meissner's *C peptone*; these are converted into *anti-peptone* and *hemi-peptone* respectively. Anti-peptone, whether the result of peptic or pancreatic digestion, once formed undergoes no further change, but remains always a peptone. The *hemi-peptone*, too, formed by peptic digestion, undergoes no further change; whilst the hemi-peptone the result of pancreatic digestion is ultimately converted into leucin and tyrosin.

Separation of parapeptone, intermediate peptone, and true peptone.—Place an ounce of solution of albumin (p. 19) in a beaker, and add an equal quantity of dilute hydrochloric acid, 0·04 per cent. (12·5 c.c. hydrochloric acid, sp. gr. 1·16, adding distilled water up to one litre), and add 20 c.c. of glycerine solution of pepsin. Digest in water-bath at a temperature of 40° C., frequently stirring. After digesting for an hour remove the beaker, and filter. Neutralize the filtrate with a solution of sodium carbonate (1 per cent.) added carefully from a Mohr's bronette. After each addition, test with both blue and red litmus-paper. When the point of neutralization is reached a fine cloudy precipitate (*parapeptone*) will diffuse through the mixture ; this must be allowed to collect at the bottom of the vessel. Filter, collect the precipitate and reserve filtrate.

A. *The precipitate* (parapeptone) collected from the filter and bottom of the beaker, must be placed on a glass slide.

Test 1. *It is insoluble in water.*—Add some water by means of stirring-rod ; the precipitate does not dissolve.

Test 2. *It is soluble in dilute acid.*—Add a few drops of dilute acetic acid, the precipitate dissolves.

Test 3. *The acid solution is precipitated by* (1) *potassium ferrocyanide ;* 2, *mercuric chloride ;* 3, *tannic acid.* —Place three distinct drops of the acid solution on a glass slide and touch each drop with one of these reagents, a precipitate in each case will appear.

B. *The filtrate*—(containing intermediate and true peptone).—Divide the filtrate into three portions (two small and one large)—reserve the larger portion. The presence of intermediate peptones is shown by

Test 1. *Precipitate with strong nitric acid.*

Test 2. *Precipitate with ferrocyanide to potassium and acetic acid.*

If intermediate peptones are present the larger portion of the filtrate which was reserved must be digested longer in water-bath till they have disappeared. Then examine for true peptone.

Test 1. *No precipitate on boiling.*

Test 2. *No precipitate with strong nitric acid.*

Test 3. *No precipitate with potassium ferrocyanide and acetic acid* (on standing a turbidity occurs).

(44) The general characters of the peptones by which their presence can be determined in solution are :—

Test 1. *Their extreme diffusibility compared with other albuminous substances.*—This can be shown by half filling two test-tubes, one (*a*) with solution of albumin, the other (*b*) with solution of peptones. Securely fasten over the mouth of the test-tubes a piece of vegetable parchment, then place the test-tubes mouth downwards in separate beakers, *a* and *b*, of distilled water. After the lapse of an hour test the water in the beakers *a* and *b* for albumin (§ 11, tests 3 and 4, and peptones § 44 respectively). It will be found that hardly any albumin has diffused out of test-tube *a*, whilst a considerable quantity of peptones will be found to have passed out of test-tube *b*.

Test 2. *A red colour is developed when a solution of peptones is treated with an equal quantity of liquor potassæ and a drop of dilute cupric sulphate is added.*—(Contrast this colour with the violet produced by the same test in a solution of albumin, and the yellow-red deposit it produces in a solution of glucose when heated).

Test 3. *Acid solutions of peptones are precipitated by bile acids* (§ 45, Test 11).

Test 4. *Solutions of peptones are precipitated by* (1) *tannic acid;* (2) *mercuric chloride;* (3) *lead acetate* (*soluble in excess*).

(45) BILE is the secretion furnished by the liver. As obtained from the gall-bladder shortly after death, it is a brownish yellow fluid of a viscid, mucilaginous nature. Its reaction is generally neutral, sometimes acid if decomposition has set; alkaline if the bile has been removed immediately after death. The specific gravity takes a wide range, from 1·012 to 1·028, the proportion of the solid matter to the water being gradually increased during digestion. The average quantity secreted daily by the human liver is calculated to amount to forty ounces; the quantity is

increased immediately after a meal, and is at its height about one hour after, and then gradually declines. Its principal ingredients are (1) Bile pigment; (2) Biliary acids combined with soda; (3) Cholesterin and fats; (4) Mucin; (5) Inorganic constituents, chiefly alkaline bases, the carbonates and phosphates of potash and soda. There can be little doubt that the principal constituents of the bile, as the conjugated acids and pigments, are separated in the liver, and do not exist pre-formed in the blood. That this is the case is shown by the fact that the blood of animals whose livers have been removed contains no trace of these substances, which it certainly would do if they were merely eliminated by this organ. Again, the blood of the hepatic artery and portal vein has been repeatedly examined, without discovering the slightest trace of either bile acid or bile pigment. From what materials of the blood the bile is formed is still a matter of conjecture; but the view generally adopted is this: that the circulating albumin, *i.e.* the albumin which has played its part in the economy and is now unfit for use, is broken up in the liver into (1) an amyloid substance or glycogen; (2) certain fatty acids; and (3) nitrogenous substances, as urea, glycocin, taurin, uric acid, and pigmentary matters. Of these substances, the glycogen is, most probably, converted into sugar, and in this form passes into the general circulation, and together with the fatty acids is oxidized to carbonic acid and water, and in this form eliminated by the lungs. The urea and uric acid likewise enter the circulation and are eliminated

by the kidneys; whilst the taurin and glycocin united to the cholic acid form the conjugated bile acids; and these, together with the pigmentary matters, cholesterin, and other fatty bodies, make up the bile which passes off by the common bile-duct into the intestinal canal. What alterations it there undergoes we do not precisely know, but it seems probable that the glyco-cholate and tauro-cholate of soda, by their decomposition furnish the free alkali necessary for the saponification of the fatty matters; and the glycocin and taurin thus set free are re-absorbed by the intestine: the cholic acid being at the same time decomposed into fatty acids of a simpler constitution. That some of the biliary substances are re-absorbed has been shown by Bidder and Schmidt, who, by analysing the fæces of dogs passed during a period of five days, found the quantity of sulphur contained in them was only $\frac{1}{15}$ of that passed originally into the intestine with the bile.

Theory of Jaundice.—When any obstruction is offered to the onward passage of the bile into the intestine, a yellow tinging of the skin, as well as of the other tissues and fluids, takes place, giving rise to the phenomenon of jaundice. In these cases the bile is taken up by the hepatic vein and carried into the circulation, and both the bile acids and bile pigments are found in the urine. In some cases, however, jaundice occurs when there is no obstruction to the onward passage of bile into the bowels, and Kühne considers that in these cases there is no re-absorption of the biliary matters, but the jaundice is produced by the colouring matter of the blood, from the introduction

of certain septic and poisonous substances into it which dissolves the blood-corpuscles, and converts their freed colouring matter into bile-colouring matter ; in these cases the bile acids are not present in the urine. Frerichs stated that when the biliary acids were injected into the blood they were transformed into bile pigments, and that the urine became darkly coloured in consequence. Kühne, on the other hand, has shown that the bile acids when injected into the blood are not decomposed, but pass unchanged out of the system into the urine ; Kühne's views have received of late general support.

The composition and general characters of bile can be demonstrated by the following tests with fresh ox-gall :—

COMPOSITION, CHEMICAL AND PHYSICAL QUALITIES OF BILE.

Test 1. *Bile acids.* (Pettenkoffer's test).—Dissolve two or three grains of grape sugar[1] in half a test-tube of water, add one drop of bile ; then run about half a drachm of concentrated sulphuric acid down the side of the test-tube at the junction of the two liquids, an intense purple rim will be developed. (N.B. As other organic substances give a similar coloration with sugar and strong sulphuric acid, when distinct evidence of their presence is required they must be separated in the manner directed in § 46, 3).

Test 2. *Bile pigment.* (Gmelin's test).—Place a few drops of concentrated nitric acid, containing traces of nitrous acid, at the bottom of a small white porcelain

[1] If grape sugar be not available, then a grain or two of cane-sugar boiled with a few drops of dilute sulphuric acid must be used instead. Care must be taken, however, not to add the concentrated sulphuric acid till the solution is cold.

dish, then float on the surface of the acid a little bile diluted with water, a beautiful play of colours ensues. The colours being developed in the following order—green, blue, violet, red, and muddy yellow. (N.B. Other pigments give a play of some of the colours, but not all, nor in the order above named.)

Test 3. *Cholesterin.*—Evaporate one drachm of bile to dryness on a porcelain dish. Treat with ether, and proceed as directed (§ 10) for preparation of cholesterin. Verify by Tests 3 and 4, § 10.

Test 4. *Mucin.*—Proceed, as directed (§ 20), for preparation of mucin from bile. Verify by Tests 2 and 3, § 20.

Test 5. *Reaction* generally neutral or alkaline. Apply red and blue litmus-paper.

Test 6. *Viscidity.*—It froths when shaken, and the lather remains persistent some time. (N.B. To show that this viscidity is caused by the mucin bile contains, precipitate the mucin with a few drops of alcohol and remove the precipitate by filtration. The filtrate will not lather when shaken.)

Test 7. *Bile does not convert starch into sugar.*—Digest an equal quantity of bile and solution of starch in water-bath at 40° C. After a short interval, test for glucose. § 3, Tests 3 and 5.

Test 8. *Bile does not convert albuminous matters into peptones.*—Digest an equal quantity of bile and solution of albumin in water-bath at 40° C. No change occurs.

Test 9. *Bile has a slight emulsionizing action on fatty matters.*—Shake up a little olive-oil with some bile in a test-tube. The oil disappears, and a milky fluid results which, under the microscope, will be seen to consist of minute globules. On standing, these globules slowly unite, and form larger ones, and finally nearly all the oil will separate itself and float free on the surface. (N B. This shows that the change was merely mechanical, and not due to chemical combination, *i.e.* the bile does not saponify the fatty matters.)

Test 10. *Bile assists the passage of fatty matters through membranes.*—Prepare two filters, moisten one with bile and leave the other dry. Fill both filters with oil. The oil in the filter moistened with bile will pass through with greater readiness than from the dry filter.

Test 11. *Bile added to a solution of peptones precipitates them.*—Add a few drops of bile to a solution of peptones; a precipitate will occur. (N.B. The reason of this is not clearly understood; it is probably analogous to the precipitation of parapeptone on neutralization with sodium carbonate (§ 43, p. 60), and is due to the alkaline bases present in bile.)

* The separation of the bile acids and bile pigment must now be undertaken; for this purpose divide the bile into two portions:

(*a*) Remove the mucus by precipitation with alcohol, filter, evaporate the clarified bile to a syrupy consistence, and mix with animal charcoal; introduce the mass whilst still hot into a flask containing alcohol, and let it digest some days; this forms the *Alcoholic Extract of Bile.*

(*b*) Free from mucus, and evaporate at a gentle heat to near dryness; this forms *Inspissated Bile.*

(46) Bile Acids.[1] 1. *Glycocholic Acid. Preparation.*—Some of the *alcoholic bile extract* is filtered; and to the clear solution an excess of ether is added, when a pulverulent white deposit will be thrown down. This is filtered off, dissolved in water, and aqueous solution precipitated with *neutral* lead acetate. This precipitate is filtered off, washed, and dissolved in alcohol, the lead removed by precipitation, with sulphydric acid, and filtered. The clear filtrate, diluted with water, on standing will deposit crystals of glycocholic acid.

Properties.—The crystals are long, delicate, colourless needles; they are slightly soluble in cold water and ether, very soluble in alcohol and boiling water; they are precipitated by the *neutral* lead acetate. Heated with an excess of baryta water, they are decomposed, forming barium cholate and glycocin. They give a deep purple colour with concentrated sulphuric acid and cane sugar (Pettenkofer's test).

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[1] Both bile acids are formed by the conjugation of taurin and glycocin with cholic acid (see Appendix to Demonstration IV.), and in the bile they are always found as sodium salts.

2. *Taurocholic Acid. Preparation.*—The mother liquor, left after the precipitation of the glycocholic acid, is to be precipitated by *basic* lead acetate ; and the precipitate treated in the same way as directed for glycocholic acid.

Properties.—Taurocholic acid never occurs in a crystalline form, but appears as an oily resinous fluid, of tawny colour, very soluble in alcohol and ether, and has a strong acid reaction ; its aqueous solution, on heating, is readily decomposed into taurin and cholic acid, and gives the purple reaction with sulphuric acid and cane sugar.

3. *To obtain the bile acids from urine.*—Evaporate the urine to a thick syrup, and treat with ordinary alcohol; evaporate this alcoholic solution, and treat the residue

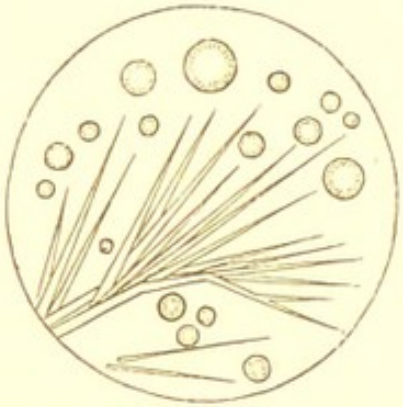

FIG. 8.—Glycocholate and taurocholate of soda from ox-bile. After J. C. Dalton.

with absolute alcohol. This solution is also evaporated, and the residue dissolved in a little water, and the solution precipitated with neutral and basic lead acetate. The precipitate is collected after being allowed to subside for twelve hours, and treated with sodium carbonate solution, and the solution filtered. The filtrate will contain sodium glycocholate and taurocholate (see Fig. 8), which give with Pettenkofer's test the characteristic reaction, when only 0·001 per cent. is present in the urine.

(47) BILE PIGMEMTS.[1] 1. *Bilirubin. Preparation.*—Extract some inspissated bile, or, better still if at hand,

[1] Other pigments are occasionally met with in biliary calculi, such as bilifuscin and biliprasin. Urobilin, which can be formed

some crushed gall-stones, successively with water, alcohol, dilute hydrochloric acid, and ether, to remove soluble matters, cholesterin, lime, &c. The dried residue is then boiled with pure chloroform, the chloroform extract is distilled to near dryness, and several volumes of alcohol added, which throws down the bilirubin.

Properties.—Bilirubin is an orange red powder, mixed with a few bluish brown crystals; insoluble in water and ether; freely soluble in chloroform, bisulphide of carbon, turpentine, and benzol; only slightly soluble in alcohol. The chloroformic solution is orange red, but loses its colour on the addition of an alkaline solution. On adding fuming nitric acid to a solution of bilirubin, a play of colours, passing from green, blue, violet, and red to a dirty yellow, takes place. (Test for bile pigments.) Concentrated sulphuric acid dissolves bilirubin, forming a brown solution.

2. *Biliverdin. Preparation.*—Is formed by passing a current of air through an alkaline solution of bilirubin.

Properties.—The green solution thus obtained deposits green flocks on the addition of hydrochloric acid, which become black when dried; the flocks are soluble in alcohol, benzol, and carbon bisulphide, insoluble in water, ether, and chloroform. According to Städeler, biliverdin is formed from bilirubin by the addition of one atom of water in the presence of oxygen; thus

$$\underset{\text{Bilirubin.}}{C_{16}H_{18}N_2O_3} + H_2O + O = \underset{\text{Biliverdin.}}{C_{16}H_{20}N_2O_5}$$

(48) Pancreatic Juice as obtained during secretion is a clear viscid fluid, free from smell, with a marked alkaline reaction. Sp. grav. 1·010–1·013. It contains: 1. Ordinary albumin, as is shown by coagulating when heated 70° C. 2. Casein, which may be precipitated by the addition of magnesium sulphate in bulk, or by the addition of acetic acid. 3. A ferment

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by heating bilirubin with sodium amalgam, has recently been shown by MacMunn to be the only one pigment present in urine which gives a well-marked absorption band with the spectrum.

which converts starch in glucose. 4. A ferment which breaks up the fatty matters into fatty acid and glycerine, and thus facilitates their saponification by the alkaline bases present in the bile and pancreatic secretion. 5. A ferment that converts albuminous substances into peptone, and by prolonged action converts some of this peptone into leucin and tyrosin. It differs from the pepsin of the gastric juice in acting in neutral and alkaline solutions only, and in the production of leucin and tyrosin as one of the final products of its action. As these bodies [1] are not present in fresh pancreatic juice, it may reasonably be surmised that their appearance after the secretion has stood some time is due to the action of this ferment on the albuminous constituents of the juice itself.

(49) TRYPSIN.—The action of the pancreatic ferments (trypsin) on starch, fat, and albumin can be studied with a glycerin solution of the pancreatic gland.

Preparation.—Remove the pancreas from an animal, killed six hours after a full meal, and after washing it, mince it into thin slices. Place the slices in a beaker, and cover with absolute alcohol. After standing two days, pour off the alcohol, and add just as much glycerine as will rather more than cover the slices. Digest for a fortnight in stoppered bottle, frequently agitating, then strain.[2]

Test 1. *It changes starch into sugar.*—Add a few drops of the glycerine extract to a small quantity of solution of

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[1] A trace of leucin, however, is always met with in the fresh juice, probably from the intrinsic action of the ferment on the albuminous matters of the secretion.

[2] Messrs. Savory and Moore supply the glycerine extract of pancreas.

starch. Place in water-bath 40° C. for a few minutes. Then test for Glucose. (§ 3, Tests 3 and 5.)

Test 2. *It emulsifies and saponifies fats.*—Take fresh olive oil free from rancidity, and which is perfectly neutral to test-paper. Place a little of this in a test-tube, and drop a small quantity of glycerine extract (if this is acid, it must be *neutralised* with a few drops of sodium carbonate solution). Both fluids, therefore, when first mixed, are neutral. Now shake, the mixture at once becomes milky. Remove a few drops, and place them under the microscope ; the oil will be found divided into extremely minute globules. Test with litmus ; the solution is still neutral or only faintly acid. Now digest the mixture in water-bath at 40° C. After a time the mixture gives a decidedly acid reaction with litmus. (The reason is, the fat has been decomposed into fatty acids and glycerine.) Now add an excess of alkali (liquor potassæ) and shake ; a soapy lather will be formed, and on examining the fluid the oil globules will be found to have disappeared. The alkali has combined with fatty acid to form a soap. (Demonstration II. pp. 11 and 15.)

Test 3. *In conjunction with dilute alkali it converts albuminous substances into peptones.*—Half fill a small beaker with a 1 per cent. solution of sodium carbonate, place in this about a drachm of finely minced muscle or fibrin, and add a drachm or rather more of the glycerine extract of pancreas. Digest in water-bath at 40° C. ; the muscle or fibrin gradually dissolves.[1]

Test 4. *A portion of the peptone so formed is converted by the further action of the pancreatic ferment into leucin and tyrosin.*—After digesting finely minced muscle as directed in the preceding experiment for about an hour, remove 20 c.c. of the mixture with a pipette. Neutralise with a few drops of dilute acetic acid ; a precipitate (peptone) is thrown down. Filter, and evaporate the filtrate to one-fifth its bulk. Treat the concentrated residue with three times its bulk of alcohol ; remove precipitate (peptone) by filtration. Concentrate the alcoholic filtrate by

[1] It is interesting to observe that the muscle or fibrin does not become translucent, swell up, and fibrillate, as was the case with gastric digestion, but remains opaque, and apparently undergoes conversion from the edges.

evaporation to one-half its bulk ; on cooling, crystals of tyrosin will separate ; collect these on watch glass (§ 30). Further evaporate the filtrate, after the removal of the tyrosin crystals, to a quarter of its bulk, and on cooling plates of leucin will crystallize out (§ 29). Now digest the original mixture for some hours longer, and remove 20 cc. and proceed as above. It will be found that the peptone precipitated by neutralisation with acetic acid and by the addition of alcohol will be less ; whilst the proportion of tyrosin and leucin obtained by evaporation of the alcoholic filtrate will be more than that obtained on the first occasion.

Test 5. *Other substances besides tyrosin and leucin are formed by the action of pancreatic juice on albumin.*— Dilute the alcohol filtrate obtained after the separation of the leucin, and divide into two portions (*a*, *b*) :

(*a*) Add drop by drop chlorine water to the diluted solution till a rose colour is developed ; this denotes the presence of *naphthilamine*.[1]

(*b*) Add a few drops of extremely dilute nitrous acid to the diluted solution ; a red colour denotes the presence of *indol*.[2]

(50) Intestinal Juice. Owing to the difficulty in isolating the intestinal secretion, it is not available for the purposes of ordinary demonstration. Indeed, very little is positively known regarding it. According to Thiry, the follicles of Lieberkühn secrete a yellowish viscid fluid of alkaline reaction, having a specific gravity of 1·011, and containing about 2·5 per cent. of solids. He obtained it by isolating a

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[1] *Naphthilamine*, $C_{10}H_7(NH_2)$, is obtained by the action of ammonium sulphide upon an alcoholic solution of nitro-napthalene, one of the products of the action of nitric acid on *naphthalene*, $C_{10}H_8$, a member of the aromatic series. It has a pungent penetrating odour.

[2] *Indol*, C_8H_7N, may be regarded as the nucleus of the indigo group. It has a peculiar odour, somewhat like that of naphthilamine, and gives the fæces their characteristic smell.

loop of intestine with a ligature below the pancreatic and biliary ducts, and collecting the intestinal juice through an artificial fistula, opening in the abdominal walls. This juice, according to Thiry, has no action on starch, fat, or ordinary albumin, but digested fibrin. Other observers have come to opposite conclusions with regard to the action of the intestinal juice on starch, maintaining that it is converted by it into grape sugar. Intestinal juice, moreover, is said to convert cane into grape sugar ; and by fermentative action into lactic acid, then to butyric acid, and finally to decompose the latter into carbonic acid and hydrogen gas.

(51) INTESTINAL DIGESTION. The food, after it has been submitted to the action of the gastric juice, passes out through the pylorus as acid *chyme*. This *chyme* consists of gastric juice holding the peptones in solution, the starchy principles of the food which have escaped conversion in the mouth, and oleaginous matters as yet unchanged. In the duodenum, the chyme is mingled with two secretions ; viz. the bile and the pancreatic fluid. These fluids decompose the oleaginous matters by emulsionising and saponifying them, in which condition they are more readily absorbed by the intestine. Pancreatic juice completes the conversion of the starch which had escaped the action of the saliva into sugar, and converts the albuminous principles into peptones and such products of decomposition as leucin, tyrosin, indol, and naphthilamine.

The action of the intestinal juice on the different principles has not yet been definitely determined.

As the fluid contents (*chyle*) of the intestine pass onward, they become more consistent, from the withdrawal of their soluble portions by the lacteals and blood-vessels; till, by the time they reach the large intestine, they contain little else but the insoluble residue of the food and the altered excrementitious matters of the bile. The contents of the small intestine gradually lose the acid reaction of the chyme, and become neutralised or even alkaline from the addition of the alkaline secretion of the bile and pancreatic juice. In the first portion of the large intestine, however, the reaction of the contents is often extremely acid, from the lactic and butyric acid fermentation of the unabsorbed saccharine matters; but in the lower part of the large intestine the acid reaction is less marked, and the mass acquires the consistence of fæces, in which form it is cast out of the alimentary canal.

(52) THE FÆCES. The fæces consist of the undigested and insoluble residue of the food, mixed with some of the products of the biliary and intestinal secretions. The quantity passed daily by a healthy adult depends much on the amount and nature of the food ingested; the average, however, may be stated at from 7 to 8 ounces. About 3 grms. represents the average daily elimination of nitrogen by the bowels. The colour of normal fæces is dark brown, due chiefly to the presence of the colouring matter of the bile. When little or no bile is passed into the intestines, the fæces acquire a pale ashy colour. Certain articles of diet or medicine impart different

colours to the fæces; thus, iron makes them black, and mercury a deep olive-green. The peculiar odour of fæces is not derived from the decomposition of the undigested residue of the food, but is due to a peculiar animal matter possessing a putrescent odour, and which is probably entirely due to the indol, the product of pancreatic digestion, and not, as supposed by some, to the specific secretion of the glands of the large intestine.

Meconium, or the fæcal-looking matter found in the intestinal canal at birth, consists almost entirely of biliary matters.

More lime than magnesia is absorbed in the intestine, since in the fæcal ash less lime and relatively more magnesia is found than in the food which has been ingested; the ratio of magnesia being as 1 : 2 or 1 : 2·5 in the fæces. The potassium salts are greatly in excess of the sodium, and this excess is increased by an abundant meat diet.

Biliary matters appear in the fæces in an altered condition, the bile pigments and acids being decomposed, the latter furnishing dyslysin, cholalic acid, and choloidinic acid.

The insoluble residue of the food consists chiefly of cells of cartilage, fibro-cartilage, fibres of elastic tissue, undigested muscular fibres, the outer envelope of vegetable cells and fibres, and partially dissolved starch granules; mixed with this undigested mass, crystals of ammonium magnesium phosphate are frequently to be recognised under the microscope.

Requirements for Demonstration V.

MATERIALS.—Saliva. Starch. Glycerine extract of Pepsin. Fresh Muscle. Fibrin. Milk. Suet. Olive Oil. Gelatin. Bile (Ox Gall). Glycerine extract of Pancreas.

RE-AGENTS.—Alcohol. Ether. Chloroform. Glycerine. Lime-water. Sodium carbonate. Acids—Acetic : Hydrochloric : Nitric : Nitrous : Sulphuric : Tannic. Solutions of, Ammonia : Ammonium oxalate : Potass hydrate : Potass ferrocyanide : Cupric sulphate : Ferric chloride : Mercuric chloride : Basic lead acetate : Neutral lead acetate.

APPARATUS.—Water-bath. Thermometer. Test-tubes. Stirring-rods. Glass slides. Porcelain dish. Red and blue litmus-paper. Filter paper and filter funnels. Animal charcoal. Parchment paper.

DEMONSTRATION VI.

NUTRITIVE FLUIDS.

(53) BLOOD. The blood is an homogeneous-looking fluid of alkaline reaction, having a saltish taste, and a faint odour characteristic of the animal from which it is drawn. Its *specific gravity* varies from 1·050 to 1·060, the average being 1·055. The quantity of water present in blood is subject to great variation. It is increased by the ingestion of fluids and by deprivation of solid food. It is diminished by exercise, and excessive action of the skin and kidneys. The fœtal blood contains less water than the maternal. Loss of blood and abstinence increase the watery elements of the blood. In some diseases, the water is diminished, as in fevers, cholera, diarrhœa, and the like. It is increased, however, in all diseases which diminish the solid constituents of the blood, as in phthisis, anæmia, leucocythæmia, and chlorosis.

The temperature of the blood varies from 36·5° C. to 37·8° C., according to the part of the body from which it has been obtained; thus, the blood of the hepatic and portal veins has a higher temperature than ordinary venous blood, and the blood of the right ventricle higher than that of the left.

The red colour of arterial and the purple colour of venous blood is due to the chemical alteration of the hæmoglobin corpuscles. Arterial blood is monochromatic, *i.e.* when viewed in thin layers by transmitted light it still retains its red tint. Venous blood, on the other hand, is dichromatic, *i.e.* the purple colour acquires a greenish tint when viewed by transmitted light. Arterial blood differs slightly from venous in containing more water and fatty matter.

When removed from the body, blood undergoes spontaneous coagulation, separating into a semi-solid clot or crassamentum, and a liquid portion or serum. This coagulation does not take place at one step. At first the blood becomes more viscid, and then assumes an appearance of jelly, and it is not till an hour or so that coagulation is complete. When the process takes place very slowly the red corpuscles have time to sink before coagulation sets in, and then a layer of colourless plasma containing an excess of colourless corpuscles forms on the upper surface of the clot and constitutes what is known as its *buffy coat*. With regard to the phenomenon of coagulation and the substances concerned in its production, see §§ 12 and 14.

APPROXIMATE COMPOSITION OF BLOOD.

Water	795	
Solids	205	
Fibrin	—	2.
Albumins		70.
Hæmoglobin		120.
Fatty matters		2.
Extractives		3.
Inorganic residue		8.

1. GLOBULIN. ORDINARY ALBUMIN AND ALKALI ALBUMIN.—Take some blood serum and dilute to ten times its volume with distilled water. Pass a current of CO_2 through it, globulin (§ 12) will be deposited. Filter. Heat filtrate to 70° C., ordinary soluble albumin (§ 11) will be coagulated and precipitated. Filter ; add to filtrate a drop of acetic acid or a pinch of magnesium sulphate in bulk, a precipitate of alkali albumin, casein (§ 16).

2. FIBRIN. Beat up some freshly drawn blood, or better still, blood flowing from an animal, with a bundle of fine twigs ; when all the fibrin is withdrawn, separate the fibrin from the twigs, place it in a muslin bag, tie the bag securely to the water tap, and allow the water to run through for some hours, from time to time removing the bag and rinsing the fibrin ; continue this till the fibrin becomes free from colouring matter. (Test according to § 14).

* ESTIMATION OF FIBRIN. Carefully clean and dry a bottle capable of holding 8 ounces, provided with a stopper, and introduce into it several strips of lead ; then weigh the whole, and after recording the weight, place in it 5 ounces of fresh uncoagulated blood, and agitate briskly for twenty minutes ; at the end of that time the fibrin will have separated and attached itself to the fragments of lead. The bottle is again weighed, to ascertain the exact quantity of blood employed, and the blood removed ; the bottle, with the fragments of lead and the adhering fibrin, are carefully washed in cold water till perfectly free from colouring matter, and then dried over a water-bath, and when thoroughly dry again weighed, the increase in weight of the bottle and lead corresponds to the amount of fibrin removed from the blood.

3. HÆMOGLOBIN. This name was given by Hoppe Seyler in 1864 to the red colouring matter of the blood contained in the corpuscles, and which separates from them in crystalline forms at a temperature of 0° C.

* *Preparation.*—Freshly drawn blood (that from the rat, guinea-pig, or dog yields the best defined crystals) is received into a saucer surrounded with ice, de-fibrinated, and a 10 per cent. solution of sodium chloride added. This mixture is allowed to stand in a beaker surrounded with ice some time till the corpuscles are deposited. The supernatant liquid is then decanted off and the mass

washed on a filter surrounded with ice repeatedly with ice-cold sodium chloride solution. When the mass is free from serum, it is to be agitated with a mixture of 1 vol. of water and 4 vols. of ether; the water dissolves the hæmoglobin, the ether, the cholesterin, and phosphorised fats.

The red aqueous solution is then filtered, received into a beaker or watch-glass surrounded by ice, and alcohol added till a precipitate begins to appear. The mixture is then set aside for some hours. If the blood used in this process be obtained from the dog, rat, squirrel, or guinea-pig, the crystals will be abundant and well defined, but in the case of the blood of man, ox, sheep, or horse the hæmoglobin is generally deposited in an amorphous state, the crystalline form being rare.

Chemical and physical properties. — The crystals of hæmoglobin are formed upon the rhombic system, the forms varying in different animals; thus, in man, though obtained with difficulty, the crystals consist of four-sided prisms with dihedral summits; in the guinea-pig the crystals are tetrahedral; in the rat tetrahedral and octohedral; in the dog and cat the crystals are needle-shaped terminated by one plane surface; in the squirrel the crystals are hexagonal.

The crystals are soluble in water and in alkaline solutions, but insoluble in alcohol, chloroform, ether, fatty oils, benzole, turpentine, and carbon bisulphide. Hæmoglobin, though a crystalloid, does not diffuse through parchment paper, thus forming an exception to Graham's theory.

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* ESTIMATION OF THE RED CORPUSCLES.—Stir a definite quantity of freshly drawn blood with the plume of a feather to remove the fibrin; add a 10 per cent. solution of sodium chloride, and set aside the mixture in a cool place till the corpuscles are deposited. The mass is then to be collected on a filter, and washed with the solution of sodium chloride till it is perfectly freed from serum; the mass is then placed in a weighed capsule and dried, the weight after drying representing the amount of corpuscles present in a certain quantity of blood.

Corpuscles are formed of a delicate membrane or *stroma*, which contains the colouring matter, hæmoglobulin, cholesterin, phosphorised fat, paraglobulin and inorganic salts, chiefly potassium

Decomposition of hæmoglobin.—Solutions of hæmoglobin readily decompose at temperatures above 0° C., and on the addition of acids, and caustic alkalis, it breaks up into *hæmatin* and *globulin* yielding about 4 per cent. of the former to 96 of the latter. With glacial acetic acid and any metallic chloride, it is decomposed into *hæmin* and *globulin*.

Hæmatin (Hoppe Seyler) is best prepared by mixing fresh defibrinated blood with a strong solution of potassium carbonate, till the liquid adhering to the separated coagulum becomes colourless. The coagulum is then dried at 50° C. and digested for some days in absolute alcohol ; the alcoholic solution after concentration will deposit rhombic crystals. *Hæmatin crystals* are of a bluish black colour with a metallic lustre, becoming brown on trituration. They are insoluble in water, alcohol, ether, and chloroform ; but soluble in acids and alkalis.

Hæmin, or hæmatin hydrochloride ; if a small quantity of blood is rubbed up with sodium chloride and boiled for a few minutes with glacial acetic acid, and the mixture evaporated to dryness and placed in a glass slide, and then a drop of acetic acid added, on warming carefully for some little time over the flame of a spirit-lamp, in the residue mixed with colourless crystals of sodium chloride, and sodium acetate, will be found rhombic tablets of hæmin ; which are of a bluish red colour when viewed by reflected, and brownish red by transmitted light. *The crystals* are insoluble in hot and cold water, in alcohol and ether. Soluble in alkaline solutions. All acids with the exception of hydrochloric and acetic acid decompose them.

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chloride and sodium phosphate. The *stroma* is the colourless portion of the living blood corpuscle, it is insoluble in water, and in sodium chloride solutions, but freely soluble in ether, chloroform, caustic soda, ammonia, and in solutions of the bile acids and urea. The stroma appears to combine with the hæmoglobin and, so to speak, fixes it, but the union is very feeble, and very slight disturbing influences set free the colouring matter. The hæmoglobin in the living blood is combined with an alkali, probably potash, to keep it in solution, as otherwise it is very insoluble and would crystallize out.

Optical Properties of Hæmoglobin and its Compounds.—It has already been stated that hæmoglobin has the power of forming a loose combination with oxygen, and in this state presenting two absorption bands in the spectrum characteristic of arterial blood. This and other changes produced by different reagents will now be more fully described. If we take a concentrated solution of oxy-hæmoglobin or undiluted blood, and place it in a tube in the slit of the spectroscope, we find the whole spectrum is obscured with the exception of the extreme red rays; on gradual dilution of the solution the spectrum clears up, allowing light to pass in the green beyond E to F, and in the blue towards G in the violet end of the spectrum; at the same time in the yellow and beginning of green between D and E, the spectrum is still obscure; as the dilution is continued the dark space disappears, leaving two absorption bands *a* and *β* with a clear space intervening; the band *a*, which is near D, is smaller and darker than *b*, which is near E (see Fig. 9, No. 1). Now if we deprive the blood or oxy-hæmoglobin of its oxygen by the action of reducing agents, as ammonium sulphide, or ammoniacal ferrous sulphate, to which enough tartaric acid has been added to prevent precipitation, being careful at the same time to exclude the air, we find the two absorption bands fade away and a single broad shadow *γ* take the place of the previously clear space; this band represents the spectrum of reduced hæmoglobin (see Fig. 9, No. 3). In the spectrum of venous blood this single band is always present, but as venous blood is never quite free from oxygen there is always some trace of the spectrum of oxy-hæmoglobin as well.

Carbon Monoxide Compound.—On passing a stream of carbonic oxide through a solution of oxy-hæmoglobin, the oxygen is displaced; at the same time, the solution acquires a dark bluish red tint, but the spectrum is only a little altered; the line *a* being shifted slightly towards E (see Fig. 9, No. 2). The absorption bands, however, do not disappear on the addition of reducing agents, and it is by this that the presence of carbon monoxide may be detected in the blood of animals poisoned by it.

Spectrum of Hæmatin.—If a little acetic acid be added to a solution of hæmoglobin the two absorption bands *a* and *b* vanish, and another absorption band appears

which covers C and extends slightly towards D (see Fig. 9, No. 4); this is the spectrum of hæmatin in an acid solution. If an alkali, such as ammonia, be added, a broad stria appears which touches C and extends nearly to D; this is the spectrum of hæmatin in an alkaline solution (see Fig. 9, No. 5). If a solution of ferrous

Absorption Spectra of the Blood and of its Colouring Matter

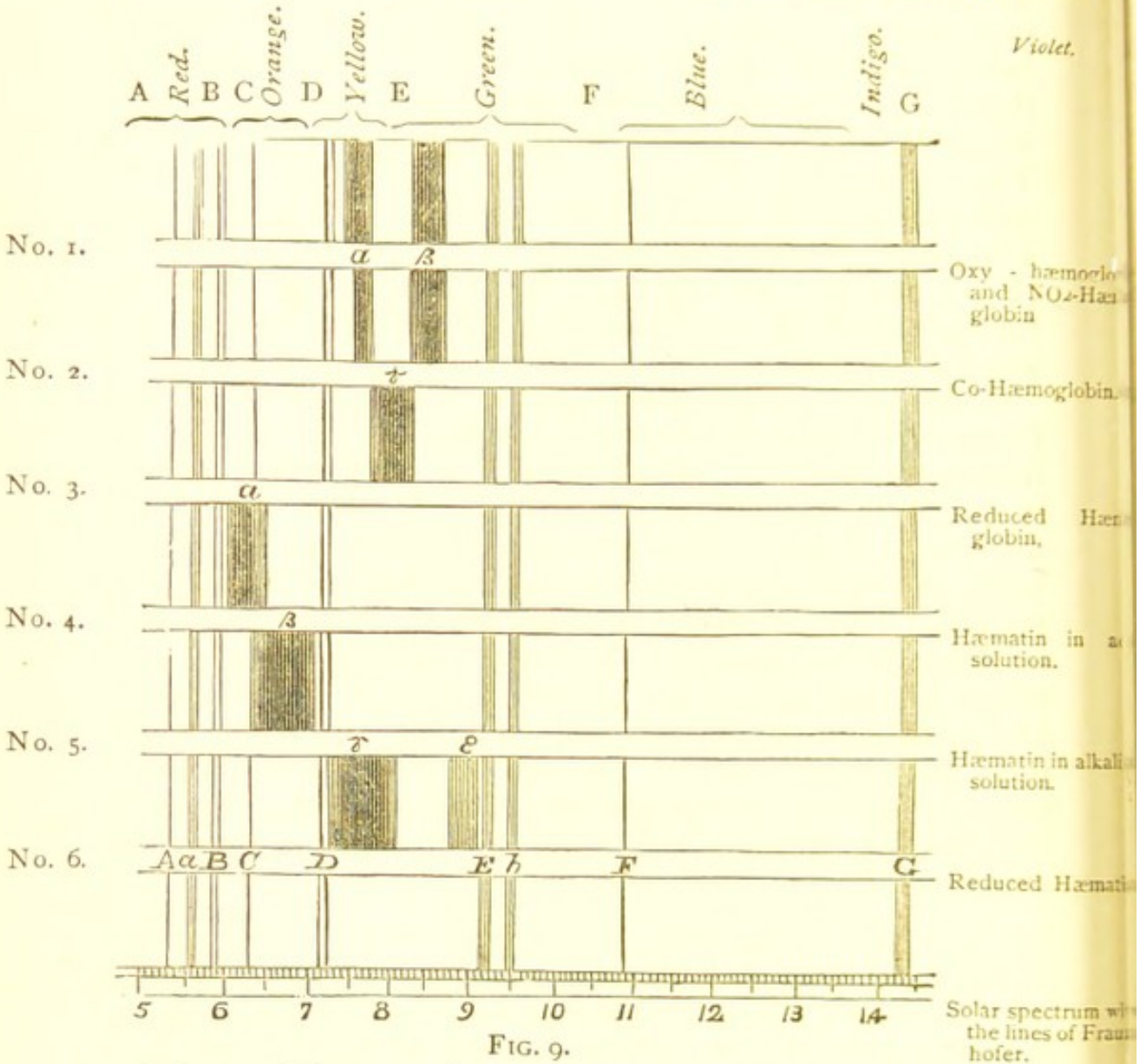

FIG. 9.

sulphate with tartaric acid and ammonia be added, the two absorption bands disappear, to be replaced by two new ones; viz., a broad band reaching from D halfway to E, and *d* a narrow band situate near E (see Fig. 9, No. 6); this is the spectrum of reduced hæmatin.

4. FATTY MATTERS.—Evaporate to dryness over a

water-bath about 50 grms. of blood; when dry, powder and introduce fragments into a small flask, digest in ether for some little time. Pour off the etherial solution into another flask, and evaporate according to directions given (§ 10, p. 16); a fatty residue will be left.

5. EXTRACTIVE MATTERS.—The residue from which the etherial solution was poured off is boiled in water for about an hour, and the aqueous solution filtered and evaporated to dryness. The residue thus obtained is heated with alcohol till nothing more is taken up by it; the alcoholic solution is then evaporated and the residue weighed, which gives the weight of *alcoholic extractives*. The residue which is not taken up by alcohol is dried and weighed and represents the *aqueous extractive matters*. The chief of these are glucose (§ 3), urea (§ 35), kreatin (§ 31), and uric acid (§ 34). The *alcoholic extractives* are hippuric acid (§ 33), leucin (§ 29), tyrosin (§ 30), and xanthin (§ 27).

6. SALTS.—Incinerate 50 grms. of blood in a muffled furnace, or over a Bunsen's burner, till all the organic matter disappears. When the ash is quite white, dissolve in 40 c.c. of distilled water and boil. Some of the ash will dissolve. Filter. The filtrate will contain the *soluble salts*, the chloride sulphates, and phosphates of soda and potash. The insoluble residue, which dissolves readily in dilute acetic acid, consists of phosphates of lime and magnesia, and iron oxide. (For quantitative determination of constituents of the ash, see Demonstration IX. under their respective heads.) The ash of blood shows a great excess of sodium over potassium salts; and of the chlorides over the phosphates, the opposite of what is found in muscle ash.[1]

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[1] GASES IN BLOOD.—These are oxygen, carbonic acid, and a small quantity of nitrogen. The process of analysis is too lengthy and complicated for the ordinary student to attempt, and will not be considered here. It is important for the student however to remember the principle on which the analysis is based. The following summary, taken from the *Handbook for the Physiological Laboratory* (Professor Sanderson, Blood, p. 194), will be useful.

In round numbers, one hundred volumes of arterial blood deliver to the Torricellian vacuum about 20 volumes of oxygen (estimated at 760 millimeters pressure and 0° temperature)

(54) MILK is the secretion furnished by the mammary gland for the nutrition of the young mammal. It consists essentially of a mixture of three substances, which represent respectively three of the important proximate constituents of the food, viz.: 1. *Casein*, the albuminous; 2. *Butter*, or oily matter, the oleaginous: and 3. *Lactose*, the saccharine element. Milk also contains a considerable quantity of *calcium phosphate*, a salt required for the ossification of the bones of the young animal.

—venous blood about 12 volumes. Of the quantity of oxygen so extracted, by far the greatest part is in combination with hæmoglobin—in other words, in the concrete state. When blood is subjected to the Torricellian vacuum, the disengagement of oxygen is complete. The blood is converted into froth, and rapidly assumes a dark colour. This appearance is due partly to the discharge of the colouring matter from the corpuscles, partly to the complete reduction of the hæmoglobin which accompanies the extraction from the *liquor sanguinis*, of its free oxygen. *Carbonic acid* gas may be extracted from arterial blood by the Torricellian vacuum in the proportion of about 35 volumes (as estimated at 760 millimeters pressure and 0° temperature) to 100 volumes of blood. Venous blood may yield 43 volumes, asphyxial blood 50 volumes. Of this quantity a certain but very varying proportion is merely absorbed, the rest is in loose combination, principally with the sodic carbonate of the plasma. It is probable that some of it is held by the bibasic sodic phosphate of the blood, and perhaps some otherwise. Hence it may be readily understood that serum contains as much carbonic acid gas as a corresponding volume of blood. When a fixed acid, *e.g.* tartaric acid, is added *in vacuo* to blood which has been already deprived of its absorbed and loosely combined carbonic acid (which together constitute what may be called its exhaustible carbonic acid), an additional quantity of carbonic acid may be obtained from it, which previously existed in the blood in the condition of neutral carbonate, principally if not entirely sodic. The apparatus for extracting the gases of blood consists essentially of two parts; a mercurial pump for removing the gases from the blood and a recipient for receiving and measuring them. The pump usually used in this country is Frankland's modification of Sprengel's pump.

AVERAGE COMPOSITION OF MILK.

	Human.	Cow's.	Colostrum.
Water	881	870	835
Casein	29	40	40
Butter	35	31	50
Sugar	50	54	70
Extractives	2	2	5
Salts	3	3	

Milk is a white or bluish white fluid, having a specific gravity of 1·018 to 1·045, and, when fresh, an alkaline reaction, due to the large proportion of alkaline phosphates and carbonates it contains, but rapidly becoming acid on exposure to the air from the conversion ot *lactose* or milk sugar into lactic acid.

After standing some time, a yellowish white stratum, the *cream*, collects on the surface, which is formed by the oily matters coming to the surface and carrying with them a portion of the casein, sugar, and milk salts; the lower portion consequently becomes poorer in quality, and acquires a more decidedly bluish tint.

Under the microscope milk appears as a turbid fluid holding a number of fat globules in suspension; these globules are about $\frac{1}{1200}$ to $\frac{1}{1300}$ of an inch in diameter, and are not dissolved, unless agitated, by alkalis or acetic acid, neither are they dissolved when agitated with ether unless caustic potash is added; from this fact it has been supposed that the globules are surrounded by a caseous or albuminous envelope.

Milk is not *coagulated* by heat at a temperature of 100° C., though a *pellicle consisting of a combination of casein with the inorganic salts forms on the surface.*

Exposed for some time at a temperature of 40° C.,

milk undergoes alcoholic fermentation, the casein converting one part of lactose into lactic acid, and the remainder into grape sugar, which is in turn converted into alcohol. It is by this means the Tartar tribes obtain their *koumis*.

Milk is *coagulated* by mineral acids, by acetic and other organic acids, and by rennet, a substance obtained from the fourth stomach of ruminant animals; the quantity of these substances necessary to produce immediate coagulation depends on the amount of casein present.

The Colostrum. The milk secreted the first week after delivery has a yellowish tint, and is richer in casein, fatty matter, and sugar than that passed at a subsequent period. Examined under the microscope, in addition to the ordinary milk globules, a number of granular corpuscles are observed, called colostrum corpuscles; they are formed by the fatty degeneration of epithelial cells lining the mammary ducts. Colostrum also contains a small quantity of albumin coagulable by heat. Specific gravity 1·045 to 1·050, and of strongly alkaline reaction.

COMPOSITION OF MILK.

1. *Alkali Albumin or Casein.*— Dilute a little milk with an equal bulk of water, and fill half a test-tube with it. Add a few drops of strong acetic acid, warm it to 40° C. in water-bath. A curd will form. Filter this off; reserve filtrate and label it *a*. Agitate the curd with ether to remove fatty matters. Pour off the ether and reserve, labelling it filtrate *b*. The curd now nearly freed from fat consists of casein. Casein is soluble in alkaline solutions. Dissolve the curd in liquor potassæ and apply tests 1, 2, 3 (§ 16), for casein.

2. *Ordinary Albumin.*—Divide filtrate *a* into two portions and neutralise with sodium-carbonate solution. 1. Heat over a spirit-lamp, coagulation occurs. 2. Run a drop of strong nitric acid down side of the test-tube to the bottom, a white zone of coagulated albumin will appear at the junction of the two fluids. (*Vide* tests 2 and 3 for soluble albumin, § 11.)

3. *Fats.*—Take filtrate *b* and place it in a small glass flask and evaporate over boiling water (taking the precaution given with respect to the evaporation of ether, p. 16, § 10). When the ether is evaporated, a few drops of oil will be left as a residue.

4. *Sugar* (*Lactose*). — Precipitate casein with acetic acid, filter. Remove ordinary albumin by heat, filter. Agitate filtrate with ether and remove etherial solution by means of a pipette. A thin whey-like fluid is left. Evaporate this to the crystallizing point; the crystals thus obtained are very impure, and must be re-dissolved by filtration through animal charcoal and re-crystallized (§ 4). Dissolve a crystal in water and apply test 5, § 3. The presence of sugar in milk can be demonstrated by diluting a few drops of milk with water, and adding either Trommer's or Fehlings alkaline cupric solution (test 5, § 3) and heating, when a red precipitate of cuprous oxide will be thrown down.

5. *Salts* chiefly consist of phosphate of lime. Evaporate 50 grammes of milk to dryness over water-bath, and incinerate the residue in a small porcelain capsule. When the ash is white, add 40 c.c. boiling water, a portion of the ash is dissolved. Filter. The filtrate consists of the *soluble salts*, chlorides, phosphates, and sulphates of potash and soda. The portion in the filter which was not dissolved consists of the *insoluble salts*, chiefly phosphate of lime. Dissolve this in dilute acetic acid. (Reserve the aqueous solution containing the soluble salts, and the acid solution containing the calcic phosphate, if required for quantitative determination, Demonstration IX., and estimate the acids and bases under their respective heads.)

* (55) CHYLE. The chyle is the fluid taken up by the lacteals of the intestinal canal, and which circulates

through the mesenteric glands and thoracic duct, and is eventually poured into the general current of the circulation near the junction of the left internal jugular and subclavian veins. In this manner the blood is constantly receiving fresh material derived directly from the intestinal canal.

If examined during the progress of digestion, chyle is a white creamy fluid of slight alkaline reaction, rich in oil globules, and, like blood, separates into clot and serum; the coagulum forming a bulky soft gelatinous clot which after exposure to the air acquires a rosy tint. The serum is turbid, and contains albumin and salts in solution; it is coagulated by heat and acetic acid.

The chyle derived from the intestinal lacteals does not contain fibrin, and consequently does not separate into clot and serum.

If the animal has been fasting, the chyle loses creamy appearance and becomes more transparent and of a yellowish colour.

ANALYSIS OF CHYLE.

	In full digestion.	Fasting.	Chylous Urine.[1]
Water	91·8	96·8	94·35
Solids	8·2	3·2	5·65
Fibrin	·2	·09	1·45
Albumins	3·5	2·30	0·78
Fats	3·3	·04	1·88
Extractives	·4	·77	1·02
Salts	·8		

[1] Analysis by author, "Case of Chyluria," in *Pathological Society's Transactions*, 1878.

The albuminous matters consist of albumin, casein, fibrin; and the peptones, as is evidenced by the readiness with which chyle passes through a filter of parchment paper.

The fatty matters consist of minute spherical globules, and form the molecular base of the chyle: their diameter is estimated at $\frac{1}{3000}$ of an inch, they disappear on the addition of ether.

Among the extractives, urea (§ 35), leucin (§ 29), tyrosin (§ 30), and sugar (§ 3), have been frequently obtained.

The ash much resembles that of blood; the sodium having a preponderance over the potassium salts, and the phosphates over the chlorides. For the separation of the above the student should proceed in the manner as directed in the examination of blood.

* (56) LYMPH is a clear, colourless, or straw-coloured transparent fluid, of alkaline reaction and saline taste; it is obtained from the lymphatic vessels and glands. In composition lymph closely resembles chyle, differing chiefly in the smaller proportion of fibrin and fatty matter it contains.

ANALYSIS OF LYMPH.

Water	. . . . .	94
Solids	. . . . .	6
Fibrin	. . . . .	·05
Albumins	. . . . .	4·28
Fats	. . . . .	·38
Extractives	. . . . .	·57
Salts	. . . . .	·72

* (57) PUS is a pathological fluid, and consists essentially of a liquid portion, "liquor puris," which is exuded liquor sanguinis, and white corpuscles or

leucocytes, which cannot be distinguished from the white corpuscles of the blood.

ANALYSIS OF LAUDABLE PUS.

Water[1]	87	
Solids	13	
Albumins		8·5
Fatty matters		3·
Extractives		0·7
Inorganic residue		0·8

1. THE PUS CORPUSCLES can be separated from the liquor puris by the addition of a 10 per cent. solution of sodium chloride, and the precipitated mass removed by filtration and thoroughly washed with the same solution till quite free from serum. The pus corpuscles or leucocytes are spherical, irregular bodies about $\frac{1}{2500}$ to $\frac{1}{3500}$ of an inch in diameter, containing a number of granules and one or more nuclei.

(*a*) Treated with dilute acetic acid, they swell up and become more transparent and the nuclei more distinct.

(*b*) Treated with ammonia or potash solutions, pus becomes tenacious and jelly-like; this character distinguishes it from mucus, which becomes less tenacious and more fluid in the addition of these solutions.

2. ALBUMINS.

(*a*) An albumin coagulable at the ordinary temperature of sero albumin, 70° C.

(*c*) Alkali albuminate or casein, precipitated by acetic acid.

(*δ*) An albumin insoluble in water, soluble in hydrochloric acid, swelling up in solution of sodium chloride (*Rovida's hyalin substance*).

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[1] The proportion of solids to the water varies of course with the nature of the pus formed; thus, in ichorous, muco- or sero-pus, the solids are diminished.

3. THE EXTRACTIVES contain urea (§ 35); leucin (§ 29); cerebrin (§ 59, p. 99); lecithin (§ 59, p. 100), and Sugar (§ 3).

4. The SALTS consist chiefly of sodium, potassium, and calcium phosphates, and carbonates with traces of iron and magnesia. Pus formed in the soft tissues contains often only a trace of calcium phosphate, but pus derived from the neighbourhood of diseased bones often contains 2·5 per cent. (For quantitative estimation, proceed as directed for estimation of salts in blood.)

Requirements for Demonstration VI.

MATERIALS.—Fresh bullock's blood, collected in deep and shallow vessels. Whipped fibrin from freshly flowing blood. (N.B. Both these can be obtained from the slaughter-house.) Blood of rat or guinea-pig for hæmoglobin. Milk.

REAGENTS.—Alcohol; ether; calcium carbonate; magnesium sulphate; sodium chloride; hydrogen peroxide. Solutions of ammonia, ammonium sulphide, ammonium ferrous sulphide with tartaric acid; alkaline cupric sulphate (Fehling's solution); magnesium sulphate; potassium hydrate; sodium chloride (10 per cent.). Acids: acetic, nitric, sulphuric, hydrochloric.

APPARATUS.—Small glass flask; pipette; spectroscope; carbonic acid gas generator; beakers; test-tubes; stirring-rods; glass slides; filters; filter-paper; red and blue litmus paper.

DEMONSTRATION VII.

SOLID TISSUES.

(58) MUSCLE. The tissue of voluntary muscle consists of a number of delicate tubes or fibrillæ, formed by a sheath of *sarcolemma* containing a semi-fluid *plasma*, with nuclei mixed up with elements of connective, adipose, vascular, and nervous tissues. The sarcolemma resembles elastic tissue in its chemical characters: it does not yield gelatin by boiling, nor is its elasticity affected by the action of acids and alkalis. The gelatin which is obtained from muscular tissue is therefore not derived from the sarcolemma, but from the connective tissue which binds the muscular fibrils together.

The muscular plasma is obtained by injecting the muscles of a freshly killed animal with a 1 per cent. solution of sodium chloride; and when the blood is thoroughly washed out, the muscles are cut up into minute fragments, frozen, and mixed with four times their volume of snow containing 1 per cent. of sodium chloride; at 0° C. the mass becomes liquid, and must

then be filtered rapidly, the filtrate at ordinary temperatures separating into

(a) *muscle clot*
(b) *muscle serum.*

Muscle clot consists of myosin (§ 13), with which is mixed a colouring matter identical with hæmoglobin, called myochrome (Thudichum). Formerly syntonin was considered as a chief constituent of the clot, but the researches of Kühne seem to show that syntonin is an artificial product, formed by the action of an acid, developed after death, on myosin and albumin.

Muscle serum contains albumin, of which Kühne recognises two varieties; viz., one coagulating at a temperature of 45° C.; and a second, the most abundant, which coagulates at 75° C. The serum also holds in solution certain extractives, as kreatin, xanthin, uric acid, sugar, glycogen, sarcolactic acid. Muscle serum agitated with ether and the etherial solution evaporated yields on an average 1·5 per cent. of fat; but this quantity is subject to great variations in the human subject, depending very much on the health and activity of the muscles in the body. In fatty degeneration olein seems to replace the more solid constituents of fat, stearin and palmitin.

By evaporation and incineration the serum yields about 2 per cent. of saline residue; in which the potash salts and phosphates greatly exceed the sodium salts and chlorides. The reaction of voluntary muscular fibre during life, and at rest, is neutral or faintly alkaline; when contracted, or after death, it becomes

acid (from the formation of lactic acid), and remains so till decomposition renders it alkaline.

COMPOSITION OF MUSCULAR TISSUE. (Kühne.)

Water	74·0	80·
Solids	26·0	20·
Albuminous substances insoluble in water, as myosin, sarcolemma, nuclei, &c.	15·4	17·7
Alkali albuminate	2·2	3·0
Gelatin	0·6	1·9
Kreatin	0·07	0·14
Fat	1·5	2·30
Lactic acid	1·5	2·30
Phosphoric acid	0·66	0·70
Potash	0·50	0·54
Soda	0·07	0·09
Sodium chloride	0·04	0·09
Lime	0·02	0·03
Magnesia	0·04	0·05

To demonstrate the chief constituents of muscular tissue, proceed as follows.

1. *Myosin.*—Kill a frog, and introduce a cannula into the aortic bulb and thoroughly wash out all the blood in the vessels by injecting a 1 *per cent. solution* of sodium chloride through it. Continue the injection till the washings become colourless. Dissect off the muscles of the legs; free them as quickly as possible from fasciæ and connective tissue, and mince very fine; wash on a filter with a strong current of distilled water till the washings give no precipitate with mercuric chloride (to remove the soluble albumin), nor an acid reaction with test-paper. The residue on the filter is then treated with a 10 *per cent. solution* of sodium chloride, and the filtrate allowed to drop into a beaker containing distilled water, when a precipitate occurs. This must be allowed to settle, and then collected on a watch-glass (§ 13).

2. *Other Albumins.*—Kill a frog and wash out the vessels with a 10 per cent. solution of sodium chloride till the washings return colourless. Mince up some of the muscles very fine, and mix with three times its bulk of distilled water ; then filter, and press the mincings nearly dry by squeezing in linen bag. Fill a test-tube three parts full of the aqueous solution and place in it a small thermometer. Heat gently in water-bath as temperature reaches 45° C. A white film forms in liquid, showing *an albumin coagulable at* 45° C. Filter. Replace filtrate in water-bath and raise temperature to 75° C., another coagulation will occur, denoting *an albumin coagulable at* 75° C. (§ 11). Filter, add a drop of acetic acid, a flocculent precipitate will fall, denoting *alkali, albumin, or casein* (§ 16).

3. *Extractives.*—The chief of these are *Kreatin, Lactic Acid, Inosite,* and *Uric Acid.* To separate these, take 200 grammes of finely chopped meat, and mix it with an equal weight of water. Heat to 75° C., and remove albumin as it coagulates. Press the coagula and return the expressed fluid to the mother liquid. When all the albumin is completely coagulated and removed, the liquid must be set aside, and filtered when cold.[1] To this aqueous extract *basic* lead acetate is added till a precipitate is no longer thrown down. The precipitate is removed by filtration and reserved for the separation of *Uric Acid* and *Inosite.* The filtrate contains *Kreatin* and *Lactic Acid.* Divide the filtrate into two portions—*a* and *b*. *To separate Kreatin:* Evaporate filtrate *a* to a syrupy consistence, and set aside in a shallow dish, when crystals of kreatin will be deposited (§ 31). *To separate* the *Lactic Acid,* the filtrate *b* must be evaporated to one-fifth its bulk and then filtered. To the filtrate baryta is added, and the precipitate removed by filtration. To the filtrate add a few drops of strong sulphuric acid, and gently distil the mixture. The residue left after distillation is then to be shaken with alcohol and allowed to digest. After standing some days it is filtered, and the filtrate mixed with milk of lime and evaporated to dryness. The residue is dissolved in water and a stream of carbonic acid gas passed through the solution, which is to be

[1] This aqueous extract of muscle can be made equally well by dissolving 1 oz. of Lieb ig's extract of meat in 6 oz. of water.

heated to 100° C. When the solution is cold, the precipitate is removed by filtration, the filtrate evaporated to dryness, the residue dissolved in rectified alcohol, and the alcoholic solution concentrated and set aside; in a few days characteristic crystals of calcium lactate will be deposited (§ 24). *To separate Uric Acid:* Suspend the precipitate resulting from the addition of the *basic* lead acetate which was reserved, in distilled water. Decompose the lead with sulphydric acid. Filter. Concentrate filtrate to one-third its bulk and allow it to stand in a shallow dish for some time; crystals of uric acid will be deposited (§ 34). Filter. *To separate Inosite:* The mother liquor from which the crystals of uric acid have been removed by filtration is still further concentrated, so as to remain permanently turbid when treated with an equal volume of alcohol. It is then heated until the turbidity disappears, and allowed to stand one or two days. The crystalline mass thus obtained is purified by re-crystallization, and then subjected to the tests of nitric acid, ammonia, and calcium chloride (§ 5, p. 10).

4. *Fats.*—A few fibres of muscle, carefully freed from all adhering external fat, minced up very fine on a glass slide and a few drops of ether added by means of a stirring-rod, and rubbed well with the muscle tissue. The etherial solution on evaporation will leave a greasy residue.

5. *Salts.*—Mince up very finely 100 grammes of muscle. Place in a porcelain crucible and incinerate over a Bunsen's burner or in a muffle furnace till the ash is quite white. Add 100 c.c. of boiling water; a large portion of the ash is dissolved. Filter. The filtrate contains the *soluble salts*, the chlorides, sulphates, and phosphates of potass and soda. Reserve the filtrate for quantitative analysis if required. The portion that was not dissolved by the boiling water consist of the *insoluble salts*, chiefly phosphates of lime and magnesia. Dissolve these with 10 c.c. of acetic acid, and add distilled water to 100 c.c. and reserve, if desired, for quantitative estimation (Demonstration IX.).

(59) Nervous Tissue and Brain Matter. Nervous tissue consists of two elements—nerve fibre and nerve

vesicle. *Nerve fibres* are found in the nerves and in the *white matter* of the brain and spinal cord. During life they appear as minute, homogeneous, cylindrical, oily-looking filaments of $\frac{1}{1500}$ to $\frac{1}{20000}$ inch in diameter. After death a separation of their contents takes place, and then can be distinguished an outer membrane of fine elastic tissue, *neurilemma*, which does not yield gelatin by boiling, and the elasticity of which is not impaired by the action of acids or alkalis. This investing membrane forms a tube containing a semi-solid fatty matter, *the medullary substance*, soluble in ether, which refracts light strongly, and gives the fibre its dark outline under the microscope; it is composed of a mixture of fat and albumin, the latter probably allied to alkali-albumin or casein. In the centre of the fibre, surrounded by the medullary substance, is the *axis cylinder*, a solid filament insoluble in ether, and receiving a pink stain from a solution of carmine; it is composed almost entirely of albumin allied to fibrin and myosin, containing but little fat. The reaction of the nerve fibres during life and in a state of inaction is neutral or faintly alkaline; on the application of a stimulus they become acid, and are always so after death, till decomposition sets in. *Nerve vesicles* which form the *grey matter* of the brain and spinal cord consist of minute cells of variable form and size, formed by a sheath of neurilemma, containing a mixture of fatty and albuminous substances, with a nucleus and a nucleolus and some granular matter. The vesicular matter contains more water and less fat and inorganic

residue than the white or fibrous matter. An aggregation of white with grey matter forms the brain and spinal cord, and other ganglionic centres, the chemical composition of which may be stated as follows:

ANALYSIS OF CEREBRAL MATTER IN 100 PARTS.

Water	80
Fats	5
Albumins	7
Extractives and Salts . . .	8

The amount of water varies considerably in different parts of the brain, thus the corpus callosum contains 70 per cent. whilst the cortical substance of the hemispheres contains 84. The spinal cord also contains less water than the brain; in the nerve centres of the fœtus, the child, and the old man there is more water than in those of adult life. The quantity of water is increased in fatty degeneration of these centres.

The fatty matters appear to be mixtures of lecithin, oleophosphoric acid, and cerebrin. Cholesterin forms 20 per cent. of the fatty matters of the brain.

The albuminous substances have not yet been satisfactorily determined. The albumin of the axis cylinder is supposed to resemble fibrin and myosin; it is however insoluble in a solution of potassium nitrate and in dilute acids. The albumin of the medullary substance is soluble in dilute acids, and resembles casein.

Extractives.—The aqueous and alcoholic solutions yield elasticin, kreatin, leucin, xanthin, hypo-xanthin, lactic and uric acids.

The salts obtained by incineration amount to 2 per cent., of which the phosphates form by far the largest proportion.

To demonstrate the presence of the chief constituents of nervous tissue [1] proceed as follows :

1. *Ordinary Albumin.*—Rub up some brain matter with cold water ; filter. Heat filtrate to 70° C., a coagulum of ordinary albumin (§ 11) will form.

2. *Alkali Albumins or Casein.*—Filter off the coagulated albumin, and divide filtrate into two portions, *a* and *b*. To *a* add a few drops of acetic acid, a precipitate will fall which is not redissolved on adding excess of acid. To *b* add a pinch of magnesium sulphate in bulk, a precipitate will fall. *Vide* Tests for Casein, § 16.

3. *Cholesterin.* — Rub up some brain substance with ether ; evaporate cautiously according to directions given § 10, p. 16. The white residue left is cholesterin, verify by Tests 3 and 4, § 10.

4. *Oleophosphoric Acid* is obtained by treating etherial extract of brain substance with ether; and to this etherial solution add some dilute sulphuric acid to remove the alkaline bases with which the oleophosphoric acid is combined ; the excess of acid is removed by repeated washing with water and the etherial solution evaporated. The dry residue being dissolved in boiling alcohol, the alcoholic solution on cooling deposits oleophosphoric acid, which must be purified by repeated washings with ether and alcohol. Oleophosphoric acid is insoluble in cold absolute alcohol. Agitated with alkaline solutions it is decomposed, yielding oleates, phosphates, and glycerin. Boiled for some hours in water, it separates, on cooling, into two layers, the upper one containing olein.

5. *Cerebrin.*—Brain pulp is coagulated by heat ; the coagulum treated with boiling alcohol, and the alcoholic solution filtered whilst hot. The precipitate which forms on cooling is separated and digested with a large quantity

[1] Fresh ox-brains obtained from the slaughter-house will furnish ample material for a large class.

of ether for some days ; the precipitate is then redissolved in boiling alcohol, and on cooling cerebrin is deposited in a pure state. Cerebrin occurs as a white amorphous powder, without taste, insoluble in water and *ether*, freely soluble *in boiling alcohol* ; neutral reaction.

6. *Lecithin.*—Agitate brain pulp with a mixture of alcohol and ether, and afterwards filter. To the clear solution, add some alcoholic solution of platinic chloride containing a slight excess of hydrochloric acid ; separate the yellow precipitate of lecithin platino-chloride by filtration. Decompose the precipitate with sulphydric acid, which liberates the lecithin hydrochlorate ; treat this with moist silver oxide, which removes the chlorine ; the lecithin silver compound can now be decomposed with sulphydric acid, when silver sulphide will be formed and lecithin left as a waxy mass. A mixture of lecithin and cerebrin constitute *protagon*, a viscous substance which dissolved in boiling alcohol is deposited in minute acicular crystals when the liquid cools.

7. *Neurin or Cholin.*—Brain pulp is digested with alcohol, and the alcoholic solution precipitated with ether ; and the etherial solution evaporated. The residue is boiled for some hours with baryta water and the excess of baryta removed by precipitation with carbonic acid. The filtrate is concentrated, mixed with absolute alcohol and filtered ; and the filtrate neutralised with hydrochloric acid, and some solution of platinic chloride is added, when a yellow crystalline precipitate of cholin platino-chloride is thrown down ; this is separated by filtration, dried, and treated with moist silver oxide, the cholin then separates as a syrupy liquid.

Salts obtained by incineration amount to 2 per cent., of which the phosphates form by far the largest proportion. For quantitative estimation proceed as directed for estimation of salts in muscle § 58, No. 5.

(60) Bone is formed of a gelatinous matrix impregnated with calcareous salts, the quantity of which varies in different bones, and at different ages. In certain diseased conditions as rachitis, mollities ossium, and caries, the calcareous constituents are diminished.

The following table represents approximatively the relative proportions, of the various constituents in health and in disease, in 100 parts.

	Healthy[1] Bone (adult).	Rickets[2] (children).	Caries[3] (adult).
Water and organic matter	33·3	65·1	55·1
Calcium phosphate . .	51·0	29·4	33·9
Calcium fluoride . . .	2·0	not stated	not stated
Calcium carbonate . .	11·3	4·3	7·6
Magnesium phosphate .	1·2	0·9	0·3
Sodium chloride . . .	1·2	0·3	3·1

1. ORGANIC MATTER.—A fragment of bone digested for 36 hours in dilute hydrochloric acid has all the inorganic matters removed, leaving the animal matrix *ossein* intact; this after long boiling is converted into gelatin (§ 21, Tests 1 and 2). *Fatty Matters.*—Agitate some crushed bone with ether, and evaporate the etherial solution, fat will be left as a residue.

2. INORGANIC MATTER.—Incinerate some powdered bone in the muffle furnace to burn off the animal matter, and divide the residue into three portions.

(*a*) Dissolve in a little dilute hydrochloric acid and neutralise with ammonia; a precipitate of *earthy phosphate* is thrown down.

(*b*) Boil with a little dilute sulphuric acid in a test-tube; the inner surface of the glass will become eroded from the liberation of hydrofluoric acid, from the *calcium fluoride.* (N.B. This test often fails.)

(*c*) Boil with some water and filter, to the filtrate add a few drops of silver nitrate solution; a

[1] Analysis by *Berzelius* of femur.

[2] Mean of three analyses of the tibiæ of three rachitic children, *Lehmann.*

[3] Analysis of carious vertebræ by *Valentin.*

white precipitate, indicating the presence of *chlorides*, will be thrown down.

(*d*) Fresh powdered bone treated with dilute hydrochloric acid effervesces, and with the acid solutions ammonium oxalate gives a precipitate of calcium oxalate; this test indicates the presence of *carbonic acid and lime.*

* QUANTITATIVE ESTIMATION OF BONE.—About 50 grammes of bone intended for analysis should be thoroughly cleaned from adhering fat periosteum and other impurities, and then reduced to powder by rasping. Place 20 grammes in a platinum capsule, the weight of which is previously determined, and dry over a water-bath till it ceases to lose weight. The loss of weight it experiences from evaporation represents the amount of *water* in 20 grammes (for general directions as to weighing and evaporation *vide* Demonstration IX., Quantitative Analysis). Now place the platinum capsule containing the powdered bone in the muffle furnace till it is completely incinerated; again weigh, and the loss of weight will represent the amount of *animal matter* in 20 grms. (for directions as to incineration *vide* Demonstration IX., Quantitative Analysis). Now take 20 grammes of powdered bone, agitate with ether, pour off etherial solution into a weighed platinum capsule and evaporate to dryness, the increase of weight in the platinum capsule will give the amount of *fat* in 20 grammes of bone. Now take the remainder of the bone, 10 grammes, and add it to that from which the fat has been removed, and also add to it the incinerated portion and thoroughly reduce the whole to a white ash. Treat the ash in the manner directed for the quantitative estimation of the salts in muscle, and proceed to determine the various acids and bases volumetrically after the method described under their respective heads in Demonstration IX. By this means the amount of water, animal matter, fats, in 20 grammes, and inorganic constituents in 50 grammes, will have been determined, from which it will be easy to ascertain percentage composition.

Requirements for Demonstration VII.

MATERIALS.—Liebig's Extract of Meat. Frogs. Ox Brains. Dried Bones.

REAGENTS.—Alcohol. Ether. Calcic hydrate. Magnesium sulphate. Moist Silver Oxide. Solutions of Ammonia, Baryta hydrate, Basic lead acetate. Calcium chloride. Alcoholic solution of Platinic chloride. Silver nitrate. Sodium chloride (1 and 10 per cent. solution). Mercuric chloride. Acetic acid. Hydrochloric acid. Nitric acid. Sulphuric acid. Sulphydric acid.

APPARATUS. — Test-tubes. Litmus Paper. Filter paper. Filters. Water-bath. Thermometer. Carbonic acid gas Generator. Glass slides. Stirring-rods. Porcelain Crucible. Muffle furnace or Bunsen's Burner. Platinum foil.

DEMONSTRATION VIII.

URINE.

(61) URINE. The urine is the principal channel provided for the elimination of nitrogen, about fifteen grms. of which on an average are passed into the urine in the twenty-four hours in the form of urea, uric acid, hippuric acid, &c. The secretion of urine depends on two factors, (*a*) blood-pressure in the renal capillaries; (*b*) the activity of the renal epithelium. Experiments show that when the amount of urinary matter in the blood is about the same, the secretion of urine goes on in proportion to the difference between the pressure in the blood-vessels of the kidneys and that in the ureters, and that no secretion takes place when the blood-pressure in the vessels sinks below 40 mm. of mercury. When the ureters are filled with mercury, a pressure of 10 mm. causes a sensible diminution in the flow, and the secretion is entirely arrested by a pressure of 60 mm. Variation of pressure in the renal arteries may be in-

dependent of that in the systemic arteries generally; contraction or relaxation being caused by the influence of the vaso-motor centres, or by morbid conditions of tissue of the kidney itself. Our knowledge with regard to the activity of the renal epithelium is not as yet very definite, though recent researches appear to point to the conclusion that the epithelium cells share in the secretion of urine, since it cannot otherwise be explained, how, when steps are taken to keep the blood-pressure constant in the renal vessels, the injection of certain urinary constituents increases secretion, unless these substances act as stimuli to the renal epithelium. The various conditions that influence the quantity of urine passed in health in the twenty-four hours may be thus briefly stated. More urine is passed in cold than in hot weather. The ingestion of fluids, or any of the urinary constituents, as sodium chloride, urea, &c., increases the amount of urine. So also does the ingestion of highly animal food, from its containing one of the principal elements of urinary excretion; viz. nitrogen. Children pass more urine proportionately than adults; and men more than women. In old age the secretion is diminished. Exercise, by increasing the pulmonary and cutaneous exhalation, diminishes the water of the urine. In disease we find it increased in cases of hypertrophy of the left ventricle; during the earlier stages of chronic Bright's disease, and in certain neurotic conditions, as after hysterical paroxysms, in diabetes mellitus, and diabetes insipidus. It is diminished during stages of shock or collapse; in all

febrile affections; after severe diarrhœa; in acute inflammatory affections of the kidney; in the last stage of Bright's disease, and in cases of considerable obstruction of the urinary passages.

PERCENTAGE COMPOSITION OF HEALTHY HUMAN URINE.

Water		960 c.c.
Solids		40 grms.
Urea	30	
Uric acid	0·5	
Hippuric acid	0·8	
Kreatinin	0·8	
Organic acids[1]	0 2	
Pigment and mucus	0·4	
Phosphates, soda, potash	1·2	
Phosphates, lime and magnesia	0·8	
Chlorides of soda and potash	4·1	
Sulphates of lime and potash	1·2	
Iron, Silica, Fluorin	traces	

1. QUANTITY. The mean average quantity of urine passed by a healthy adult may be stated at 50 fluid ounces or 1450 c.c. in the twenty-four hours. As the twenty-four hours' urine is required for the quantitative estimation of many of the constituents of that secretion it is important that the collection be carefully made. The following points must be attended to.

The patient must be directed to empty his bladder, and the hour of his doing so noted. This sample is rejected. The next sample after this must be stored in the receiving jar (fig. 10[2]), which is a large glass bottle 12 inches high and

[1] These acids consist of phenylic, damaluric, damolic, taurylic, and kryptophanic acids; also a fatty acid which is probably palmitic.

[2] These jars, as indeed all the apparatus required for urinary analysis, can be obtained of Griffin and Sons, Garrick Street, Covent Garden.

5 inches wide, with wide ground mouth 2½ inches bore covered with a ground glass plate to exclude dust. It holds about five pints or three litres, and is graduated into spaces of 100 c.c. Every succeeding sample of urine must be placed in the jar, and the quantity, specific gravity, and reaction of each noted and recorded. The patient must empty his bladder exactly at the end of the twenty-four hours.

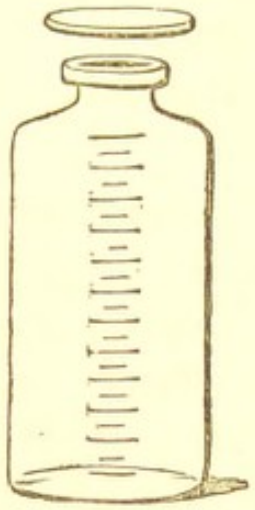

FIG. 10.

2. SPECIFIC GRAVITY.—SOLIDS.—The collected urine of the twenty-four hours in a normal condition gives a specific gravity of 1·020, and contains about four per cent. of solid matter ; therefore as fifty fluid ounces represents the daily urinary secretion, the solids passed out of the body by this channel in the twenty-four hours will amount to 2 ozs. ; or if French measures be employed, to 58 grms. The specific gravity of urine is usually taken by a special instrument called a Urinometer. These are spindle-shaped instruments, consisting of a bulb filled with mercury, a float filled with air, and a long narrow stem containing a graduated scale, from 1·000°, the point at which it floats when plunged in distilled water, up to 1·045°, about the highest point attained by human urine. The urinometer is floated in the urine, and the depth at which it settles denotes the specific gravity, which is then read off from the scale. The urinometers supplied by instrument makers are often unreliable, when accuracy is required it is advisable to test and graduate for oneself, in standard solutions at a given temperature. It must also be remembered that every difference of 4° C. (7° F.) from the temperature at which the instrument was graduated represents a difference of one degree in the registration of the urinometer. Where a series of daily observations are made with regard to the specific gravity of the urine in any given case, it is advisable to always use the same urinometer, and either to record the temperature at the time of observation, or else make the correction for the variation. When greater accuracy is required, the density must be determined by actually weighing a given bulk of the liquid measured at the standard temperature. The weight compared with that of an equal quantity of water

denotes the specific gravity. The most convenient quantity of liquid to weigh is 50 c.c. Bottles holding 50 c.c. when filled up to a mark on the neck of the tube, may be obtained at the instrument dealers. A cubic centimeter of water being a gramme, such a bottle holds 50 grammes of water, but more or less of liquid lighter or heavier than water. As liquids expand and contract by alteration of temperature, the measurement of the liquid must be performed at a certain degree. 15° C. is most convenient. A bottle (Fig. 11), which holds 50 grammes of water, when measured at 15° C., having been obtained, the liquid to be examined is poured in; if the temperature is too low, it is raised by plunging the bottle into hot water, if too high, it is lowered by plunging it into ice water. When the proper degree is obtained we take the weight of the bottle, filled with the liquid; and the weight, after that of the empty bottle is subtracted, multiplied by 2, gives the specific gravity in hundreds.

FIG. 11.

The actual proportion or urinary solids to the water can only be accurately determined by evaporating a known weight of urine and ascertaining the weight of the residue (Quantitative Analysis, Demonstration IX. § Exp. 2.) The process is tedious, and could not advantageously be employed when examinations had to be made daily. For clinical purposes therefore the solids may be calculated from the specific gravity, and if this be taken with the precautions mentioned above, will yield sufficiently accurate data. The calculation of the urinary solids can be effected by Trapp's formula,[1] which consists in multiplying the two last figures of the specific gravity of the urine by a constant number, 2 in the case of urines with a specific gravity below 1·025, and 2·3 in urines above. The total represents the solid matter in grammes in 1000 c.c. of urine. For example: a person passes 1450 c.c. of urine in the twenty-four hours and the specific gravity

[1] Trapp's formula is obtained from the following consideration:—Normal urine contains 4 per cent. of solid matter, and the specific gravity of the collected urine of 24 hours is 1·020. Multiplying the two last figures of this specific gravity by 2, we get 40 in every 1,000 parts, or exactly 4 per cent.

of this urine is 1·020, then multiplying the two last figures of the specific gravity by two, we have 40 grms., the amount of solid matter in one 1000 c.c. of urine; and the $\frac{1450 \text{ c.c.} \times 40}{1000} = 58$ grms. of solid matter in the twenty-four hours. (N.B. This is an example of normal urine, of normal quantity and normal specific gravity.) Again, a patient passes 3600 c.c. of urine of a specific gravity of 1·035. Then by multiplying the two last figures of the specific gravity by 2·3 we obtain 80 grammes as representing the solids in 1000 c.c. of the urine; and then $\frac{3600 \text{ c.c.} \times 80}{1000} = 288$ grms. (N.B. this is an example, and by no means an extreme one, of the increase of solids over the normal in a case of diabetes.)

3. COLOUR.—Human urine is best described as amber coloured, that is yellow with just a tinge of red. The pigment that imparts this colour to urine has been the subject of much discussion, and considerable differences of opinion regarding it exist.[1] In the present unsettled state of the question it has no practical bearing, and therefore the nature of the pigment need not be considered here. But as variation of the natural colour of the urine is an important sign giving the physician indication as to the nature of many diseases, the colour of the urine should be noted in all clinical records. The following table will give the student a useful basis for comparison.

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[1] Dr. G. Harley has isolated a pigment which he calls *urohæmatin*. Dr. Schunk has isolated two pigments which he calls *urian* and *urianin* respectively. Dr. Thudichum gives the name *urochrome* to the colouring matter of the urine, and which, under various processes of decomposition, yields *uropithin*, *uromelanin*, and numberless other products. Whilst Dr. MacMunn has recently shown that *urobilin* is the only pigment present in urine which gives a well-marked absorption band with the spectrum.

Colour.	Indication.
1. Pale. Colourless to straw-colour.	Low specific gravity (diabetes mellitus excepted). Abundant. Passed in health after large draughts of fluid. In disease observed in anæmia, chlorosis, diabetes. Rare in febrile diseases, usual during convalescence from them. Seldom very acid; frequently neutral or alkaline.
2. Normal. Golden yellow, or amber.	Negative.
3. High coloured. Reddish yellow to red.	High specific gravity. Scanty. Passed in health after abundant meat meals, or severe exercise. Common to febrile diseases. In hectic often gives more certain indications of intensity of febrile increase than is afforded by pulse and temperature. Usually very acid.
4. Dark coloured. Smoky, brownish, porter coloured.	Presence of abnormal pigment. (*a*) *Pathological.* Blood (p. 127). Bile (p. 128). Brown and even black urine frequently observed in cases of melanosis and splenic disorders. (*b*) *Accidental.* Colouring matter introduced into the body with food or medicine, as blood red from beetroot, dark colour from carbolic acid and salicylic acid.
5. Various colours. Green, blue, and indigo.	Pathological and diagnostic indication not determined. Considered due to the oxidation changes of indican—a substance which exists in extremely minute quantities in normal urine and which in some conditions may become increased and altered chemically.

4 Reaction.—The freshly collected urine of the twenty-four hours in a normal state has an acid reaction, though individual samples collected at various periods of the day may be neutral or even alkaline. The acidity of the twenty-four hours' urine is equivalent to about two grms. of oxalic acid; that is, it requires so much sodium

hydrate solution to neutralize it as would be required to neutralize two grms. of oxalic acid. The acidity of the twenty-four hours even in health is subject to considerable variations. The hourly variations in health are tolerably constant, and occur with tolerable regularity, so that authors speak of the increase and decrease of the acidity as the *acid and alkaline tide*. The acidity is most marked in the urine secreted at night and before meals, whilst there is a marked decrease after eating and other physiological stimuli. The following table gives the average result of several observations made by myself to determine the ebb and flow of these tidal variations in the acidity :—

Time.	Total acid as oxalic acid.	Acidity per hour as oxalic acid.
11 P.M. to 8 A.M.*	1·14 grm.	0·12 grm.
8 A.M. to 11 P.M.	·21† ,,	0·07 ,,
11 A.M. to 1 P.M.*	·40 ,,	0·20 ,,
1 P.M. to 4 P.M.	·11† ,,	0·03 ,,
4 P.M. to 7 P.M.*	·29 ,,	0·09 ,,
7 P.M. to 11 P.M.	·07† ,,	0·02 ,,

From this table it will be seen that after breakfast acidity was depressed five degrees, and gradually rose again after the influence of the morning meal had passed away till just before lunch. After lunch it falls again but rises before dinner, and falls again in the post-prandial hours. This close connection of depression of the acidity of the urine after the ingestion of food has led observers to attribute the phenomenon to that cause. Dr. Bence Jones believed that the depression in the acidity of the urine was caused by the withdrawal of acid from the circulation to supply the acid for the gastric secretion. Dr. Roberts of Manchester believes the depression to be caused by the entrance of newly digested food into the blood, and as he thinks the normal alkalinescence of the blood is due to the preponderance of alkaline bases in all

* Breakfast at 8.30 A.M. ; lunch at 1 P.M. ; dinner at 7 P.M.

† These samples were sometimes neutral, rarely alkaline.

ordinary articles of diet; a meal *pro tanto* is a dose of alkali which must for the time add to the alkalescence of the system and consequently of the urine. But it these views that depression of acidity occurs without food being ingested at all. Dr. Hermann Weber some years ago observed that whilst breakfast decidedly had happens unfortunately for the complete acceptance of an influence in lowering the acidity, yet when he went without breakfast the acidity was still lessened, though not to so great a degree. This observation I have been able to confirm. The use of the cold douche, sweating in the vapour-bath, a small quantity of strong tea or coffee after fatigue, all induce a marked depression in the acidity of urine. No explanation has hitherto been offered to account for this, but it is probably to be found in the fact that the act of rising, cold bathing, the vapour bath, tea and coffee, promote the elimination of carbonic acid from the lungs and thus lead to decrease of acid in the system.

The acid reaction of the urine is due to the presence of *acid sodium phosphate*, which is generally considered to result from the decomposition that occurs between an acid or an acid salt and neutral sodium phosphate in the blood. Now one of the chief acid salts in the blood is undoubtedly bicarbonate of potash and soda, an acid salt with an alkaline reaction. The neutral phosphate which is also present in the blood has also an alkaline reaction. The decomposition which results between these two salts may be represented as follows :—

Bicarbonate.	Neutral Phosphate.	Carbonate.	Acid. Phosphate.

$$NaH_2CO_3 + Na_2HPO_4 = Na_2HCO_3 + NaH_2PO_4.$$

Morbid urine has frequently an alkaline reaction which is produced either by the presence of *fixed alkalies* of the phosphates and carbonates of potash and soda, or from *volatile alkali*—ammonia. In the former case the blue reaction on litmus-paper is permanent, in the latter the blue reaction disappears when the test-paper is dried. Alkalescence due to excess of the alkaline carbonates of potash and soda is a condition frequently observed after the ingestion in large quantities of vegetables and fruits,

some associated with a form of simple dyspepsia. The alkalescence due to excessive elimination of the phosphates of the alkaline bases is a condition not yet thoroughly understood; it is frequently met with in individuals of neurotic tendency, and it sometimes precedes and accompanies phosphatic diabetes. Urine alkaline from volatile alkali or ammonia is generally associated with disease of the urinary passages. Recent experiments have shown that even when the ammoniacal ferment is introduced into the bladders of healthy animals the urine does not become ammoniacal till inflammatory actions begins in the mucous membrane of the bladder. (Quantitative Estimation of Free Acid in Urine; see Demonstration IX. § 64 Exp. 1).

(A.) Variations of Normal Constituents of Urine.

5. Urea.—This is the characteristic constituent of the urine. In health, as we have stated in § 35, p. 46, the excretion of urea is proportionate to the nitrogenous food ingested. In disease, however, this relationship often disappears. In all febrile affections and other acute diseases which are accompanied by rapid disintegration of tissue, the quantity excreted is largely increased, and this increase is closely connected with the rise of temperature, indeed often precedes it.[1] In these cases the increased rise does

[1] The close connection between urea and temperature can be well followed in ague, as the following table, showing hourly rate of excretion, proves :—

	Dr. Ringer, *Med. Chir. Trans.* 1859.		Author, *Med. Times*, January, 1876.	
	Temp.	Urea.	Temp.	Urea.
Before shivering .	98·2– 99·8	1·36 grm.	98·2	1·56 grm.
Shivering . . .	99·8–103·4	2·17 ,,	103 –105·3	4·36 ,,
Hot stage . . .	103·4–102·4	1·28 ,,	105·3–103·4	1·87 ,,
Sweating . . .	102·4– 98·6	0·93 ,,	103·4– 98·3	1·68 ,,
Remission . . .	—	—	97·2– 98·3	1·19 ,,

not come from food, for it is out of all proportion to it, but from the breaking up of the fixed albumin of the muscles, nerves, &c. Cases are recorded in which there was excessive elimination of urea unaccompanied by pyrexia. In these cases the excess noted was three or four times above the normal. Prout, who first noticed this condition, suggested it might be a prior stage of diabetes. Excessive elimination of urea is often accompanied by excessive elimination of phosphoric acid, and in these cases the increase often precedes chronic pulmonary disease, or diabetes, or is accompanied by nervous symptoms.[1] The excretion of urea is diminished in nearly all chronic affections unaccompanied by pyrexia. In diseases of the liver accompanied by considerable destruction of liver substance, as in cancer, cirrhosis, or abscess of that organ, the amount of urea excreted is generally very considerably diminished. Rapidly growing cancer likewise causes a diminution. In Bright's disease the excretion is diminished, not from want of formation, since the amount of urea found in dropsical exudation added to that obtained from the urine frequently amounts to what might be said to represent a normal quantity if passed out of the system by the proper channel. (For general description of urea and Tests, refer to § 35, p. 46, Demonstration IV.; for Quantitative Estimation, § 64, Exp. 2, Demonstration IX.)

6. URIC ACID is found in extremely small quantities in healthy urine. The variations in its amount depend less on the nature of the food than upon special conditions of the internal organs. In fever it is increased even relatively more than urea. Whenever the process of oxidation is incompletely performed in the body uric acid is found in excess; as in functional and organic disease of the liver, disease of the spleen, and in leucocythæmia. In disease of the lungs, where elimination of carbonic acid is impeded, uric acid is increased. Professor Odling suggests in these cases that the kidneys act vicariously for the lungs by getting rid of a portion of carbon that otherwise would pass off through the latter; the carbonic oxide constituent of uric acid taking the place of carbonic acid. Uric acid

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[1] *Vide* paper by Author, *Path. Soc. Trans.* vol. xxix. p. 151.

in normal urine is always in combination with bases of potash, soda, and ammonia, forming soluble salts—*the urates.* When these, or free uric acid are deposited, some abnormal condition of the system, or change in the chemical constitution of the urine, is indicated. (Urinary Deposits, § 62, Nos. 1 and 2.) For Chemical Test and physical qualities, see Demonstration IV. § 34, page 45; for Quantitative Estimation, see Demonstration IX. § 63, Exp. 3.

7. *Kreatin and Kreatinin; Hippuric Acid.*—Very little is known respecting the variations of these substances in disease, and what little is known is of no practical clinical value. They will not therefore be further alluded to here. (See Demonstration IV.—Kreatin, § 31; Kreatinin, § 32; Hippuric Acid, § 33.)

8. *Chlorides* are the most variable of the urinary constituents. In health, the quantity met with in the urine closely corresponds with the quantity of salt taken with the food. In all acute febrile diseases a diminution of the quantity of chlorine excreted occurs, at times almost entirely disappearing. In pneumonia the diminution commences with the stage of hepatization, and reappears gradually as resolution commences. Parkes says that sometimes it may be retained some days, and is then poured out in such quantities as to raise the specific gravity of the urine, although the water is increasing and the other solids decreasing. In acute rheumatism with effusion into the joints, and in exudative pleurisy, a considerable decrease is likewise noted. The decrease in these diseases is mainly accounted for by the fact that the exudation material poured out is particularly rich in chlorides. In ague during the cold and hot stages the excretion of chlorides is greatly increased. (For Quantitative Estimation, see Demonstration IX. § 64, Exp. 4.)

9. *Phosphates* are of two kinds. (*a*) The *soluble*, the alkaline phosphates of sodium and potassium which are soluble and are never deposited from urine. (*b*) The *insoluble*, the earthy phosphates of lime and magnesia which are thrown down in alkaline urine. The conditions under which the earthy phosphates are increased in disease are not well understood. It appears, however, from recent observations, that an increase is noticed (1) at the onset and accompanying many chronic diseases associated with

tissue waste, especially preceding pulmonary consumption. (2) As preceding and accompanying some forms of saccharine diabetes. (3) As being eliminated in considerable quantities, and running a course resembling diabetes, without any sugar appearing in the urine.[1] The earthy phosphates are frequently thrown down as a deposit, either simply, or associated with ammonia in the form of triple phosphate, ammonium-magnesium-phosphate—Urinary Deposits, § 62, No. 4. (For Quantitative Estimate, see Demonstration IX. § 64, Exp. 3.)

10. *Sulphates.*—The following brief summary of the conclusions arrived at by Vogel represents the state of our knowledge respecting the increase or diminution of sulphuric acid in the urine. (1) A considerable diminution indicates that the patient has taken very little food, or only vegetables without meat. (2) An habitually large excretion of sulphuric acid with excess of urea, indicates a preponderance of animal food. We are not justified in considering that increased secretion of sulphuric acid depends on abnormal decomposition of the sulphur compounds of the body except in acute febrile affections, during which little or nothing is taken in the way of food. (For Quantitative Estimation, see Demonstration IX. § 64, Exp. 5.)

(62) URINARY DEPOSITS. 1. *Uric Acid.*—Though this substance may be excreted in excess, it will not be deposited unless the urine be unduly acid. The increase in the acidity of the urine may be caused by an absolute increase of the acids and acid salts usually excreted, or relatively by a diminution of the alkaline bases, or by the formation of acid in the urine, after emission the result of fermentative changes. Uric acid deposits vary in colour—from pale fawn, amber, to deep orange-red. Under the microscope it is found to consist of crystals assuming various forms, the most common of which are smooth, transparent, rhomboidal tables of variable size; mixed with these are often seen hexagonal tables, rectangular four-sided prisms, diamond-shaped plates, and short barrel-shaped cylinders; if these mixed crystals are dis-

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[1] Report on two cases by Author, *Path. Soc. Trans.* vol. xxix. p. 151.

solved in liquor potassæ, and recrystallized by the addition of hydrochloric acid, the characteristic rhomboidal tables (whetstone shape) become more evident (Fig. 12). The deposit is not dissolved when heated, but is soluble in liquor potassæ. Moistened with nitric acid, gently evaporated, and the reddish residue touched with ammonia, it yields a magnificent violet red colour (murexide).

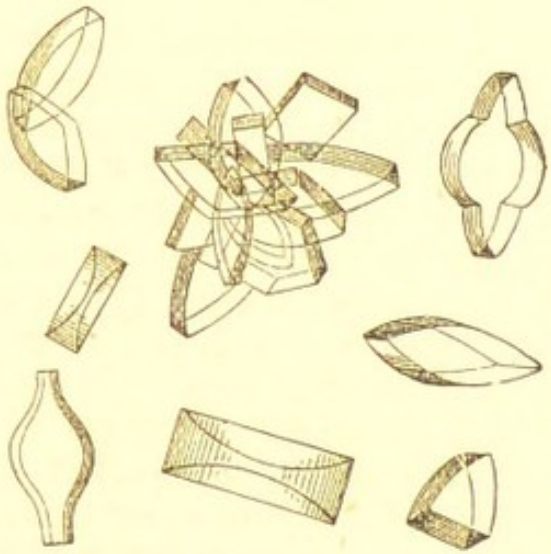

FIG. 12.

2. *Urates.*—The urates are generally deposited in a granular form, more rarely as crystals. With the exception of ammonium urate they occur in acid urine. These deposits are met with under the following conditions: (1) Diminution of the quantity of water; during febrile excitement, or after an unusual amount of exercise accompanied with profuse perspiration, the quantity of water eliminated by the kidneys is much diminished, consequently the urine is nearly saturated with the uric acid salts, which are then only retained in solution as long as the urine remains warm, and as soon as the urine cools they are deposited. A similar saturation occurs when the uric acid salts are excreted in excess, though there may not be any absolute diminution of the quantity of water. (2) The increase of acidity in the urine. The presence of free acid in the urine decomposes the soluble neutral salts of uric acid, forming acid urates, which being less soluble, are precipitated. This increase in the acidity *of the urine* may take place before or after

emission. (3) Deficiency of the phosphate of soda. A deposit of urates of nearly white colour is often noticed in pale urines of low specific gravity, and containing no excess of uric acid. The deposit in these cases has been attributed to deficiency of phosphate of soda in the urine, the urates being soluble in solutions of this substance. The colour of the deposited urates may be pale white, brick-dust red, pink, crimson, and deep purple; they usually appear under the miscroscope in small masses of amorphous granules; when deposited in the crystalline form, the *urate of soda* generally forms spherical globules from which minute spicules project. *Crystals of urate of ammonia* which are sometimes met with in alkaline urine mixed with crystals of triple phosphates (Fig. 13), are

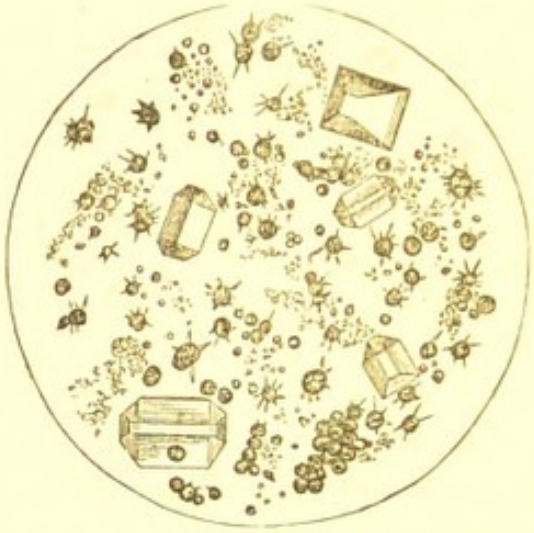

FIG. 13.

known also by their spiked globular form. Both the amorphous and crystalline deposits dissolve when heated, are soluble in alkaline solutions, insoluble in acetic acid: and give, when heated with nitric acid and ammonia, the purple reaction (murexide).

3. *Oxalates.*—Crystalline deposits of calcium oxalate are extremely frequent, they are found in two distinct classes of urines. The one of deep yellow or orange colour, of high specific gravity, often turbid with mucus, urates or phosphates; the other pale, of medium specific gravity, perfectly clear save at the bottom of the urine-glass, where a slight cloud of mucus is collected, and in which the deposited oxalates will be found. In the first instance the calcium oxalate is probably formed in the urine subsequent

to emission, from chemical changes taking place in that fluid, such as decomposition of uric acid and the urates, or by the oxidation of the pigmentary matters and mucus (acid fermentatives). In the latter case, which is less frequent, the oxalic acid undoubtedly comes from the blood ; although, as Dr. Parkes observes, it is difficult in any given case to say to what class of bodies, and to the arrest of what metamorphosis, the oxalic acid when formed in the system is to be referred. In this class of cases the patients suffer from intense depression and hypochondriasis. Crystals of calcium oxalate are recognised under the microscope as small, brilliant, square octohedra with strong refractive power ; other varieties of crystalline form are met with—as the dumb-bell, discoid,

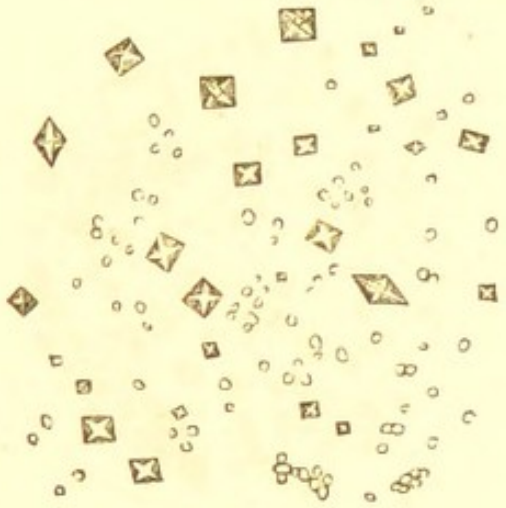

FIG. 14.

and diamond-shaped crystal, but the *letter envelope shape* of the octohedral crystals alone are characteristic (Fig. 14). The crystals are insoluble in water and acetic acid; but are soluble in dilute hydrochloric acid and strong solutions of acid sodium phosphate.

4. *Earthy Phosphates.*—The earthy phosphates of lime and magnesia are insoluble in alkaline solutions ; consequently they are deposited from urine when that secretion becomes alkaline ; their deposition therefore must not be regarded as indicating excessive elimination of phosphoric acid. The urine may become alkaline either from excess of the *fixed alkalies*, the sodium and potassium carbonates, or of the alkaline phosphates ; or from the presence of *volatile alkali*, ammonium carbonate. A urine alkaline

from *fixed alkali* deposits only calcium phosphate, either in an amorphous or crystalline form; in urine alkaline from *volatile alkali*, the triple phosphate or ammonium-magnesium-phosphate is deposited as well as calcium phosphate. Urine alkaline from *volatile alkali* or ammonia, is generally associated with disease of the urinary passages, and recent experiments have shown that even when the ammoniacal ferment is introduced into the bladders of healthy animals the urine does not become ammoniacal, till inflammatory action begins in the bladder. The ammoniacal condition of the urine met with in paraplegia is undoubtedly due to some irritative condition of

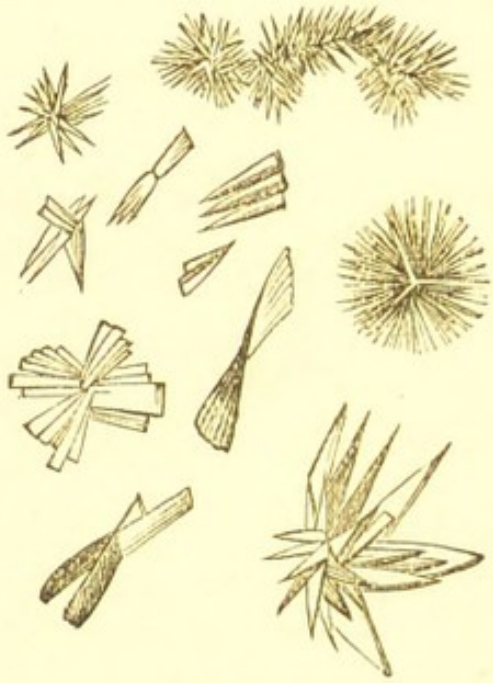

FIG. 15.

the bladder. *Calcium phosphate* as a urinary deposit appears under the microscope either in small granules or globules, or very rarely in a crystalline form. The crystals vary considerably in form and size, appearing generally as thin, twisted, acicular crystals crossing each other at right angles, or thick and wedge-shaped, or small and pointed, united to form ropes and rosettes (Fig. 15). The deposit is not dissolved by heat, but is soluble in acetic and other acids. This last distinguish them from modified crystals of uric acid, for which they are often mistaken. *Ammonio-magnesium phosphate*, or triple phosphate, is deposited in a crystalline state, the

characteristic form of which is the right rhombic prism (Fig. 16); sometimes foliaceous and penniform crystals are seen mixed with these: this change of form is supposed to be due to excess of ammonia and rapid crystallization. Mixed with this deposit are granules of calcium phosphate, and often spiked globules of ammonium urate. Heat does not dissolve the triple phosphates, but they are readily soluble in acetic and other acids.

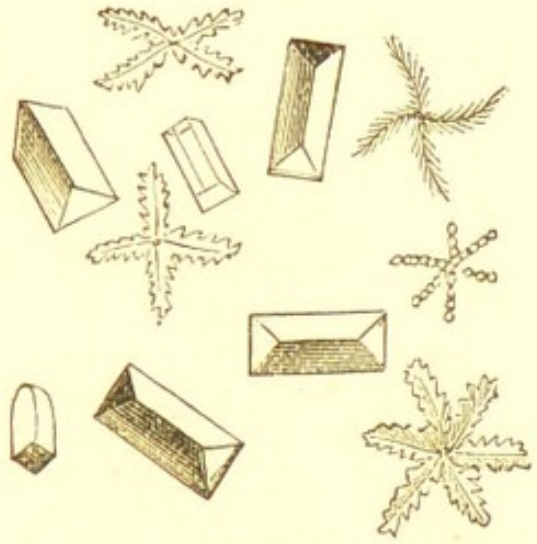

FIG. 16.

5. CYSTIN.—This substance is a somewhat rare urinary deposit, and its pathological significance has not been determined. Patients may void cystin for years and yet be apparently in good health. Cystin forms either a white or fawn-coloured amorphous sediment. (See Demonstration IV. § 28.)

6. LEUCIN AND TYROSIN.—These substances, usually associated together, are never found in normal urine, their presence in that fluid always denoting serious disease; thus, they are met with in severe cases of jaundice, in acute yellow atrophy of the liver, in cirrhosis of that organ, and in severe cases of small-pox and typhus. *Leucin* as it appears in the urine is not crystalline, but forms circular oily-looking discs which float on the surface; if these are dissolved in boiling alcohol the solution on cooling will deposit leucin in crystalline plates resembling cholesterine in appearance. *Tyrosin* forms a greenish yellow crystalline deposit. The crystals are long prismatic needles, which cluster together to form stellate groups, and are sometimes so closely aggregated as to form balls

of spiculated needles. (See Demonstration IV. §§ 29 and 30.)

7. *Deposits derived from the urinary passages.*—1. *Mucus.* The mucus derived from the mucous membrane of the urinary passages consists of a few pigmentary amorphous particles, mucus corpuscles or young epithelial cells, nucleated epithelial cells of the bladder, and in women of the vagina. This mucus when the urine is left at rest separates as a light transparent cloud which is diffused through the urine. In diseased conditions of the mucous membrane the quantity of epithelium discharged may be enormous, and is then generally mixed with pus. In these states the mucus is often deposited in narrow twisted bands or ropes not infrequently resembling cylinders from the renal tubes or prostate. Alcohol and acetic acid deposit mucin from its solutions in thread-like masses. Mucin is distinguished from the pyin of pus in not being precipitable by mercuric chloride. The following is a concise account of the microscopic and chemical characters of the various epithelial products, casts and cylinders, cancer cells, and organic corpuscles that may be met with in mucous deposits. *Pigmentary particles* have been well described by Dr. Roberts: they consist of amorphous reddish brown or orange-coloured masses sometimes enclosed in a cell wall of obliquely ovoid outline from $\frac{1}{1500}$ to $\frac{1}{1000}$ of an inch in diameter; they keep badly in urine and disappear when decomposition sets in; no pathological significance is attached to them. *Mucus Corpuscles and Pus-cells.*—Round cells containing one or more nuclei and many granules, the nucleus rendered more visible by the addition of water or acetic acid. *Blood Corpuscles* appear as thick circular bi-concave discs, varying in diameter from $\frac{1}{3000}$ to $\frac{1}{4000}$; they contain no nucleus, they have a tendency to roll together in masses; solutions of potash, ammonia and acetic acid render them pellucid, but do not dissolve them. *Renal Epithelium.*—Rounded and spheroidal cells with well-defined nucleus which does not require the action of acetic acid to develope it; only met with in kidney disease assoicated with the renal casts and albuminous urine (Fig. 17). *Vesical Epithelium.*—Large round cells, considerably larger than renal cells, and scaly epithelium of irregular outline; the latter in women is also

derived from the vagina (Fig. 18). *Spermatozoa* may be recognised by their peculiar form, the spherical head and elongated tail,—this latter in very acid or very alkaline urine is often curled up round the head. *Columnar* epithelium is derived chiefly from the ureters and urethra, the cells are somewhat triangular with well-defined nuclei, and

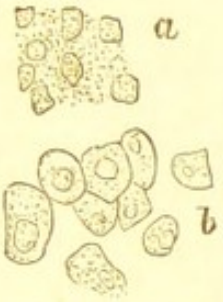

FIG. 17.

(*a*). Renal cells from tubules.
(*b*). Renal cells from pelvis of kidney.

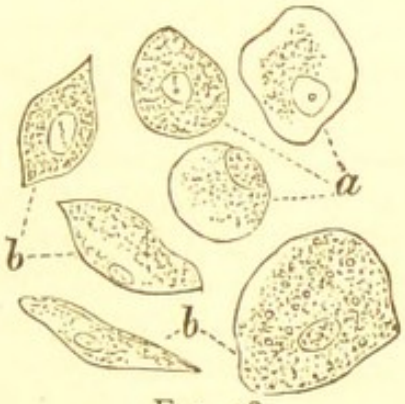

FIG. 18.

(*a*). Vesical epithelium.
(*b*). Vaginal epithelium.

generally adhere closely together ; from the elongated and tailed appearance they may be mistaken for cancer cells ; they are, Dr. Parkes observes, by no means so large, perfect, or numerous as cancer cells generally are (Fig. 19). *Cancer Cells* are generally characterised by their large size, their irregular outline, with frequently caudate extremities, and

FIG. 19.

(*a*). Columnar epithelium from ureter.
(*b*). Ditto ditto from urethra.

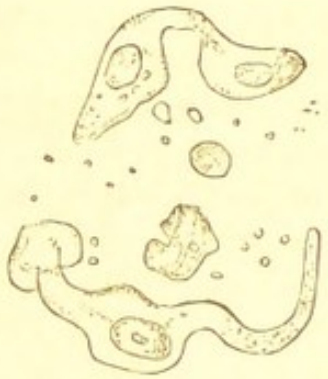

FIG. 20.

also by the prominence and magnitude of the nucleus and nucleolus ; many of the cells often appear to be undergoing fatty metamorphosis (Fig. 20.) *Renal Casts* vary in size, structure, and transparency. The most common are the simple epithelial casts which consist of a cylinder of coagulable material to which the epithelium of a urini-

ferous tubule is attached, they vary in length from $\frac{1}{150}$ to $\frac{1}{50}$ of an inch, and in breadth from $\frac{1}{500}$ to $\frac{1}{1000}$; and are met with in the early stages of kidney disease associated with blood-casts, which are fibrinous moulds covered more or less irregularly with blood corpuscles. In the chronic forms of Bright's disease the granular and waxy cast is met with; in the former the cast is covered with granular material derived from degenerated epithelium; they are more or less opaque in appearance and are about $\frac{1}{600}$ of an inch in length. The waxy and transparent casts possess a hyaline structure of so fine a character that it is difficult to distinguish them, the addition of a solution of iodine with iodide of potassium imparts a yellow colour

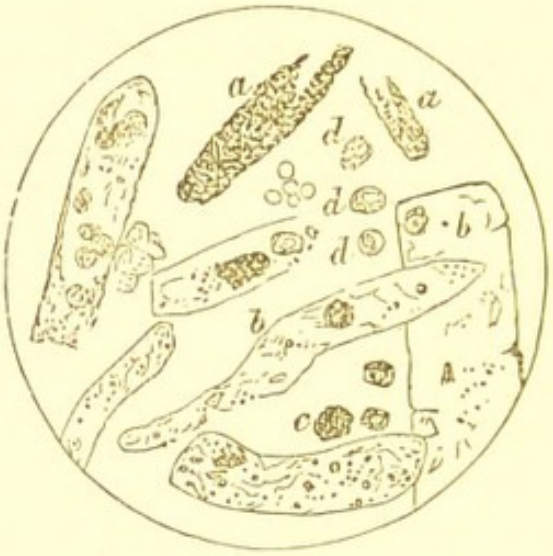

FIG. 21.

(*a*). Granular cast.
(*b*). Waxy cast.
(*c*). Fatty masses.
(*d*). Renal epithelium.

to them which makes them more distinct. Their surface is frequently marked with faint striæ, their size is extremely variable, sometimes oily particles are distributed through them, and then we have the fatty cast (Fig. 21). In addition to renal epithelium, blood corpuscles and oil globules—pus cells, uric acid, and oxalate of lime crystals, urates and crystals of triple phosphate, may adhere to the cast. Casts owing to their lightness frequently take some time before they are deposited, and if scanty in number may easily be overlooked. The urine should therefore be allowed to stand before the deposit is collected. *Prostatic Cylinders.*—Plugs composed of

aggregated pus cell mucus derived from the prostate are occasionally met with in the urine; they are flattish in shape, and much larger than renal cylinders possibly could be; they are soluble in acetic acid; similar shreds are frequently discharged from the urethra after gonorrhœa. *Cylinders of altered Mucus.*—After acid fermentation has commenced mucin may be deposited in long stringy ropes from the urine. In alkaline urine, if pus is present, the action of the alkali converts it into a stringy white mass. *Globular or pyriform coagula* are generally composed of tubercle masses, or masses of cancer cells mixed with blood corpuscles.

2. *Pus.*—Inflammation in any part of the genito-urinary tract may furnish pus. The most frequent source in men is from the urethra, and the vagina in women. Very slight catarrh of the bladder is sufficient to produce pus cells, in severe cystitis the quantity of pus discharged is very great; the tendency of the urine to become alkaline and the consequent ropy condition of the urine, together with the enormous quantity of pus discharged, sufficiently indicate the source of the deposit.

In pyelitis the quantity of pus discharged is usually less than when derived elsewhere; the urine retains its acidity, and though there may be no actual hæmorrhage recognisable by the unassisted eye, yet under the microscope blood corpuscles will be frequently recognised in the deposit, also the columnar epithelium derived from the pelvis of the kidney. Sudden discharges of pus generally proceed from the emptying of an abscess in some part of the urinary passages. Pus is deposited, in acid and neutral urines, in a dense greenish creamy layer; the deposit is not acted upon by acetic acid. The addition of liquor potassæ or ammonia causes it to assume a jelly-like condition; this reaction distinguishes it from mucus. Purulent urine is always albuminous, and therefore the existence of clap, gleet, and leucorrhœa should always be inquired for before assuming the existence of kidney disease. The pus corpuscles may be recognised under the microscope as spherical, irregular shaped, indistinctly granular vesicles; varying from $\frac{1}{2500}$ to $\frac{1}{3000}$ of an inch in diameter, containing one or more distinct nuclei. The addition of water or acetic acid causes the corpuscles to become pale and swollen, and at the same time more transparent, and

the nuclei are more distinctly visible. Pus corpuscles cannot be distinguished from lymph corpuscles, mucus corpuscles, and other young cell forms.

(B.) Abnormal Products met with in Morbid Urine.

1. *Albumin.*—The presence of sero-albumin in urine was till lately regarded as pathognomic of Bright's disease; but clinical experience has shown that albumin appears in urine under a variety of conditions, so that we cannot certainly, from its mere presence, declare that there is organic disease of the kidneys without taking other circumstances into consideration, such as the presence of casts and the persistent nature of the albuminuria. (N.B. In some forms of chronic renal disease albumin may be absent for some days together from the urine.) Temporary albuminuria is met with under a variety of conditions. In what may be considered as a fairly normal condition of the system it has been observed after excessive ingestion of albuminous substances, after unwonted and severe exercise, after nervous excitement, and even after cold bathing. In other conditions it is met with in connection with malaria, in obscure forms of dyspepsia, and in certain forms of blood-poisoning. (For Tests[1] for Albumin, see Demonstration III. § 11, Tests 3, 4, 5; for Quantitative Estimation, see Demonstration IX. § 63, Exp. 4.)

2 *Blood* may be derived from any portion of the genito-urinary tract. Urine containing blood varies in colour from a slight smoky tint to a deep-red or chocolate-brown, according to the quantity mingled with it. In artificial solutions one part of blood added to 1,500 parts of urine imparts a decidedly smoky tint, one part in 800 gives a

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[1] In testing for albumin it should be borne in mind that undue acidity or an alkaline condition of the secretion will convert the sero-albumin into acid or alkali albumin respectively, and then the test by heat and nitric acid will fail. Should there be any doubt, *neutralize* the urine with dilute solution of sodium carbonate if acid; and add *dilute* acetic acid *slightly in excess* if and then proceed to test with heat and nitric acid.

bright cherry-red, one part in 400 a chocolate-brown.[1] Urine containing blood is albuminous. In fresh urines and with undissolved blood the presence of blood corpuscles deposited on standing will be sufficient to determine its presence. If decomposed, Heller's test will detect very small quantities of blood in solution; he directs the urine to be boiled and concentrated liquor potassæ added, and then again boiled; the phosphates are deposited, carrying with them the colouring-matter of the blood. This deposit must be removed by filtration and dried, and the residue rubbed up with sodium chloride and boiled with a few drops of glacial acetic acid and again evaporated to dryness, and the dried residue placed on a glass slide and examined with the microscope; very small quantities of blood will yield hæmin crystals. If blood be present in extremely minute quantities and the corpuscles dissolved, it can alone be detected by examination with a microspectroscope (see Blood, Demonstration VI., pp. 81 and 82), when the characteristic bands of hæmoglobin, hæmatin and acid, and alkaline hæmatin respectively will be observed. The guaiacum test is quite unreliable, since globulin, casein, mucin, &c., will develope the blue reaction when blood is absent.

3. *Fat, Cholesterin.*—The most common source of fat in the urine is from degenerated epithelium of the renal tubules in Bright's disease and from pus. Rare cases have been recorded of fat in the urine after taking large quantities of cod-liver oil. In chyluria fat globules have been sometimes observed; generally only minute granules could be detected, probably fat in an extremely minute stage of subdivision. Fat globules under the microscope present the form of flattened discs with a dark and irregular outline; ether dissolves them, and if the etherial solution be evaporated on a glass slide a greasy residue is left. *Cholesterin* is occasionally met with; it may be mistaken for leucin, but can be distinguished from this substance by its solubility in ether. (Separation and Tests for Cholesterin, see Demonstration II. § 10.)

4. *Xanthin, Cystin, Leucin, Tyrosin.* (See Demonstration IV. §§ 27, 28, 29, and 30.)

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[1] See paper by Author, *Lancet*, May 20th, 1873.

5. *Sugar.*—Healthy urine contains a minute trace of sugar; this quantity may be temporarily increased in various pathological conditions not yet well understood; when the quantity is more or less permanently increased the disease known as diabetes is indicated. Diabetic urine is usually passed in large quantities, is of higher specific gravity and of a paler greenish-yellow colour than healthy urine. On exposure to air it becomes covered with a white scum, which under the microscope appears as minute oval sporules, generally joined together so as to form an irregularly-jointed conifervoid stem; this growth is known as Torula cervisæ. (For Tests for Sugar, see Demonstration I. § 3, and for Quantitative Estimation, see Demonstration IX. § 64, Exp. 7.)

6. *Bile.*—Urine containing bile is of a deep brown-yellow colour, and imparts a yellow stain to linen rags dipped in it. (For theory of Jaundice and Tests for Bile Acids and Bile Pigments, see Demonstration V. §§ 45, 46, and 47).

7. *Fungi and Entozoa.* — Stale decomposing urine speedily develops a number of organisms, the chief of which are *vibriones*, small moving filaments about $\frac{1}{3000}$ of an inch long — *Pencilium glaucum* or mildew. The sporules are oval, nucleated, and vary greatly in size. According to recent experiments these sporules removed from the urine, washed, dried at 55° C., on again being moistened will revive, and then will decompose dilute solutions of urea. The thallus is derived from sporules either by elongation or budding, and is an elongated, branched, cellular shoot, which rises to the surface of the urine, and gives off branched tufts of sporules. *Torula cerevisiæ*, or yeast plant, is always present in saccharine urine. In the earlier stages of its growth it cannot be distinguished from the preceding; the fully-developed thallus, however, bears a brownish-coloured spherical head of sporules. *Sarcinæ* have been frequently found in urine. The cells of the urinary sarcinæ are much smaller than those of the stomach and lung. They have been met with in the pelvis of the kidney, but the chief seat of growth is probably the bladder.

Entozoa.—The following are the chief: Echinococcus hominis, Strongylus gigas, Bilharzia hæmatobia, and Pentastoma denticulatum. (For particular descriptions of

each the student is referred to the ordinary text-books of medicine.)

8. *Accidental Constituents of Urine.*—Hair, fœtal bones, fæces, &c., may find their way into the urine from communication of cysts and or intestines with the urinary passages. In addition to these, the substances that may be introduced intentionally to deceive are innumerable. Usually the imposture is easily detected; but sometimes a most searching investigation is required, and the substances introduced have to be submitted to a complete analysis before the medical man can be quite sure he is not dealing with some rare urinary deposit.

(C.) URINARY CALCULI. The size, colour, and general appearance must be noted. Also whether on section they are made up of concentric layers, or present an uniform surface. A portion of the stone is then broken down and reduced to powder, and if made up of different layers, a portion of the powder of each layer must be taken and submitted to analysis. The nucleus in all cases should be examined.

1. ANALYSIS OF THE ORDINARY VARIETIES OF URINARY CALCULI.

Powder a small portion of the calculus, divide the powder into two portions and place one at one end and the other at the other end of a glass slide. Label them respectively A and B.

(A.) *Soluble in Liquor Potassæ.*—The powder labelled A is touched with a drop of liq. potassæ added by means of a stirring-rod. It dissolves (or only partially dissolves if there are traces of phosphates and oxalates present); a drop of HCl added to the solution causes a white precipitate of

1. URIC ACID. { Chars under blowpipe, leaving little residue. Gives murexide reaction with nitric acid and ammonia (§ 34, p. 45).

2. URATES. — Chars under blowpipe, leaving considerable residue. The urates are soluble in boiling distilled water, while uric acid is not. Dissolve in boiling distilled water; filter and evaporate. The residue if urates are present will give murexide reaction.

(B.) *Soluble in Hydrochloric Acid.*—Touch the powder labelled B with hydrochloric acid, it dissolves without effervescence[1] (or partially dissolves if there are traces of uric acid). The acid solution gives a white precipitate when touched with ammonia, and indicates,

1. PHOSPHATE OF LIME. — Chars but slightly under blowpipe, leaving friable white ash, *infusible.* Ash dissolves *without effervescence* in dilute HCl. The acid solution gives a white gelatinous precipitate with ammonium oxalate solution denoting lime; also a precipitate with uranium nitrate denoting phosphoric acid.

2. AMMONIUM-MAGNESIUM-PHOSPHATE, OR TRIPLE PHOSPHATE. — Chars slightly, leaving greyish ash which *slowly fuses.* Soluble in HCl *without effervescence*, from which excess of ammonia throws down a characteristic crystalline precipitate of triple phosphate; *vide* p. 121, Fig. 16.

3. PHOSPHATE OF LIME WITH AMMONIUM - MAGNESIUM - PHOSPHATE. (Syn. *Mixed phosphates* or *fusible calculus.*) — Chars slightly, the ash *fuses readily* into a porcelain-like mass soluble in dilute HCl *without effervescence.* Acid solution gives a white precipitate with ammonia consisting of amorphous phosphate of lime with crystals of triple phosphate.

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[1] If there is effervescence it denotes the presence of carbonate of lime, traces of which are often found in phosphatic calculi.

4. Oxalate of lime.[1]		Chars considerably under blow-pipe, leaving a white ash which dissolves *with effervescence* in HCl. A fragment of the calculus will not dissolve in oxalic acid.

2. Rarer Forms of Urinary Calculi.

These are Xanthin, Cystin, and Fatty substances.

1. *Xanthin Calculi* are extremely rare ; they are usually smooth and of a cinnamon colour, taking a polish when rubbed. They dissolve in nitric acid without effervescence, and do not yield the murexide reaction with uric acid and ammonia. (For general description of Xanthin and Tests see Demonstration IV. § 27.)

2. *Cystin Calculi* are also rare ; they have a smooth surface, a greenish-yellow colour, and break with a crystalline fracture; they are the softest of all calculi, and for some days after their removal are compressible. (For general description and Tests see Demonstration IV. § 28.)

3. *Fatty Calculi*, composed of mixture of fat, mucus, and withered cell-forms, and termed urostealith, are detected by their solubility in ether.

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[1] Oxalate of lime is frequently met with in phosphatic calculi ; in these cases the ash effervesces with dilute HCl, denoting the oxalate. And a portion of the calculus dissolved in acetic acid will give a whitish precipitate on addition of uranic nitrate solution ; and if triple phosphate is present the addition of ammonia to the HCl solution will give characteristic crystals (p. 121, Fig. 16).

DEMONSTRATION IX.

QUANTITATIVE ANALYSIS.

(62) QUANTITATIVE ANALYSIS. A substance present in a mixture may be estimated in two ways.

1. By precipitating, collecting, and weighing the precipitate; this is the GRAVIMETRIC METHOD.

2. By precipitating or otherwise altering it with a *solution* of a reagent of known strength, and ascertaining the quantity of the reagent required to effect the complete change; this is the VOLUMETRIC METHOD.

In quantitative analysis the French or Metric system of weights and measures is employed. In this system the *gramme* is taken as the *unit of weight* which represents a cubic centimetre of distilled water, at its greatest density; viz. 4° C.

The *unit of capacity* is the *litre*, which contains 1,000 cubic centimeters; consequently a litre of distilled water weighed at 4° C. should weigh 1,000 grammes.

The *multiples of these units* are characterised by Greek prefixes : thus,

Gramme . .	1.	Litre . . .	1.
Decagramme .	10.	Decalitre . .	10.
Hectogramme .	100.	Hectolitre . .	100.
Kilogramme .	1000.	Kilolitre . .	1000.

The unit of each denomination being ten times as great as the preceding one. The *sub-multiples* of the units are characterised by Latin prefixes ; thus,

Milligramme .	·001	Millilitre . .	·001
Centigramme .	·01	Centilitre . .	·01
Decigramme .	·10	Decilitre . .	·10
Gramme . .	1·	Litre . . .	1·

Here the unit of each denomination is ten times less than the one below it.

The following tables give the French Measures of Weight and Capacity, and their English equivalents calculated out to many places of decimals :—

MEASURES OF WEIGHT.

(Dr. Warren De La Rue.)

	English grains.	Troy ounces.	Avoirdupois lbs.
Milligramme	0·015432	0·000032	0·0000022
Centigramme	0·154323	0·000322	0 0000220
Decigramme	1·543235	0·003215	0·0002205
Gramme	15·432349	0·032151	0·0022046
Decagramme	154·323488	0·321507	0·0220462
Hectogramme	1543·234880	3·215073	0·2204621
Kilogramme	15432·348800	32·150727	2·2046213

MEASURES OF CAPACITY.

	Cubic inches.	In pints.	In gallons.
Millilitre	0·061027	0·001761	0·00022010
Centilitre	0·610271	0·017608	0·00220097
Decilitre	6·102705	0·176077	0·02200967
Litre	61·027052	1·760773	0·22009668
Decalitre	610·270515	17·607734	2·20096677
Hectolitre	6102·705152	176·077341	22·00966767
Kilolitre	61027·051519	1760·773414	220·09667675

For all practical purposes, however, it is sufficient to remember that

1 gramme	= nearly	15·4 grains Eng :
1 kilogramme	= „	2 lbs. 3 ozs. 5 drs.
1 litre	= „	1 pt. 15 fl. oz. 2 drs.
1 decilitre or 100 c.c.'s	= „	3 fl. oz. 3 drs.
1 cubic centimetre	= „	16·3 minims.

(63) GRAVIMETRIC ANALYSIS. *The balance* usually employed is enclosed in a case fitted with glass doors (Fig. 22). It carries 100 grammes, and indicates a variation of $\frac{1}{5}$ milligramme or $\frac{1}{300}$ grain ; this variation is ascertained by the pointer moving decidedly one side or other of the central mark on the register ; when in equilibrium it vibrates an *equal* distance on each side of the central mark. When at rest, or whilst the weights are being adjusted, the instrument is kept at rest by means of a contrivance which steadies the pans ; this contrivance is worked by a screw outside the case. *The rider* (*b*)—to weigh below ·01 grms. we use the rider ; this is formed of bent wire capable of being shifted to any divisions in the beam—the rider being placed on the last, or tenth division, is equal to ·01 grm. ; while each division nearer the fulcrum

makes a difference in weight of ·001 grm. less. The rider is caught up and moved by a brass rod worked outside the case (*a*).

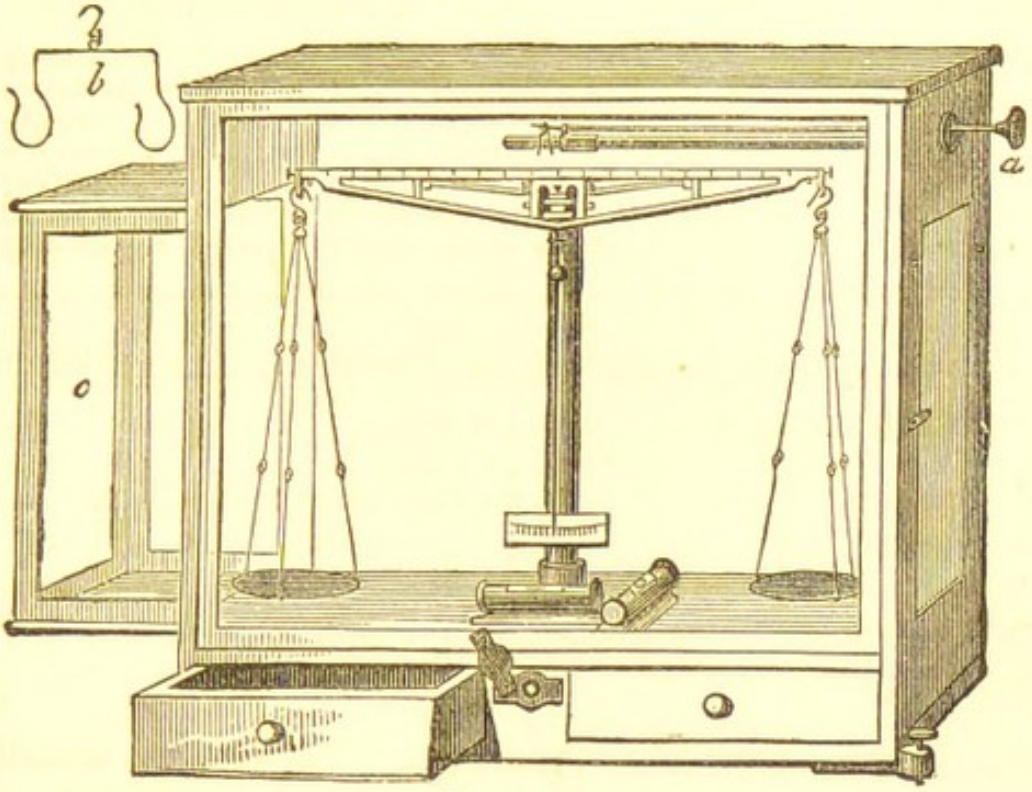

FIG. 22.

The process of weighing will be best described by an actual example.

EXAMPLES OF GRAVIMETRIC OPERATIONS.

EX. 1. *Weighing.*—Suppose we wish to determine the weight of a platinum basin. We first of all ascertain if the balance is in equilibrium by liberating the pans with the screw and observing if the pointer swings to an equal distance on each side the central mark on the scale; if it does not swing to an equal distance but moves to one side more than the other, the balance must be adjusted. A brass arm about two inches long fixed to a screw above the fulcrum serves for this purpose. A deflection to either side makes that the heaviest.

The pans are steadied and the basin, perfectly cleaned and dried previously in an air-bath, is placed in one pan,

usually the one on the left hand of the operator, and the weights placed in the other; at first we guess at the probable weight, and place say fifty grammes on this pan; this proves too much, for the pointer travels towards the pan supporting the basin. We remove the 50 and try the next highest or 20 grammes; as this is too little we allow it to remain and add the two 10 gramme weights, thus making 40 grammes in all. This being still insufficient we add 5 grammes; still too little; allow it to remain and add 2 grammes; too little; add 1 gramme, too much; remove the one gramme weight and try ·5 gramme; too much; try ·2 gramme, too little; allow ·2 gramme to remain and add ·1 gramme; too little; add another ·1 gramme; still too little. As we had previously tried the ·5 gramme weight and found it too heavy, we recommence with ·05 gramme; this proving too little is allowed to remain and ·02 gramme added; still too little; ·01 gramme, too much; remove and recommence with rider say on 5; too much; on 3; this is just sufficient, the pointer swings an equal number of degrees on either side of the scale. The weight of the basin is therefore 47·453 grammes.

Ex. 2. *Estimation of the Water, Organic Matter, and Salts.*—Take a clean platinum capsule, and having weighed it by the above process, place in it a definite quantity of the substance to be determined, say 50 grammes of blood, milk, or finely minced bone or muscle, and evaporate[1]

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[1] Evaporation Water-bath.—For ordinary purposes a cheap and efficient water-bath can be made from an old oil can, by fitting it with a cork perforated to admit an ordinary glass funnel. The can is filled with water and the platinum capsule placed in the open part of the funnel, the escape of steam being allowed by a folded piece of paper placed between the edge of the filter and the capsule. The apparatus is supported on an ordinary retort stand and ebullition maintained on an argand gas-lamp placed below. The evaporation requires about two hours. Five or ten minutes must elapse after the water appears dissipated, to allow the residue to become thoroughly dry. The basin is then removed, the bottom wiped quite dry and allowed to cool under a glass shade in which is placed some oil of vitriol in a beaker, in order to prevent absorption of moisture. It is then weighed. The difference is usually very small, and varies from ·02 grm. to ·1 grm.

over a water-bath till it ceases to lose weight. On weighing when cold the loss will represent the amount of water withdrawn from 50 grammes of the substance; for example, if the united weight of the capsule and 50 grammes of the substance is 110 grammes, before evaporation, and only 70·5 after, the substance will have lost 39·5 grammes of water; and $\frac{39{\cdot}5 \times 1000}{50} = 790$ grammes the quantity of water in 1000 parts.

The residue left in the platinum capsule, and which by the above weighing was found to weigh 70·5 grammes, is introduced into a muffle furnace or over a Bunsen's lamp, and incinerated till all the organic matter disappears; this residue is again weighed, and its weight represents the amount of inorganic residue in 50 grammes of the substance. If, for example, after incineration the weight of the capsule and residue is 60·4 grammes, then 10·1 grammes of organic matter has been burnt off, and 0·4 gramme represents the inorganic residue of 50 grammes of the substance, therefore $\frac{0{\cdot}4 \times 1000}{50} = 8$ grammes the inorganic residue in 1000 parts of the substance.

Ex. 3. *Determination of Uric Acid in Urine.*—Collect the urine passed in the twenty-four hours and measure. Take 200 c.c. and add 20 c.c. of strong hydrochloric acid. Set aside in a tall urine-glass for twenty-four hours to allow the uric acid crystals to separate. Dry a small filter paper in the air-bath at 120° C. and weigh. Collect the crystals on this filter and wash them well with water slightly acidulated, using a wash-bottle. Dry them with the filter in the air-bath and weigh. For example, the weight of the dry filter is 0·27 gramme, with the crystals when dried it weighs ·42 gramme, therefore the weight of the crystals in 200 c.c. of urine will be 0·15 gramme, and if the quantity of urine passed in twenty-four hours be 950 c.c.: then,

Quantity of urine taken.		Weight of uric acid in 200 c.c. of urine.				Weight of uric acid in 950 c.c.
200 c.c.	:	0·15	::	9·50	:	0·7

Ex. 4. *Estimation of Albumin in Urine.*—Collect the urine for twenty-four hours and measure; introduce 50 c.c. of this urine into a Mohr's burette and allow it to fall a c.c. at a time, into a porcelain dish containing an ounce of boiling distilled water. If the urine is sufficiently acid of itself no further addition of acid will be required, but if not it will be necessary when all the urine has been passed into the boiling water, to add a few drops of dilute acetic acid to the mixture, most carefully avoiding excess. When the albumin is completely coagulated, it is allowed to settle at the bottom of the vessel before proceeding to filtration. When the supernatant fluid is quite clear, it is poured upon a *weighed* filter;[1] the coagulated albumin remaining on the filter whilst the fluid runs through; any particles of albumin adhering to the porcelain dish are to be removed with a feather and placed on the filter. The mass is then well washed with boiling water till the washings give no precipitate with silver nitrate solution. The filter with the mass is now removed and placed in a watch glass and carefully evaporated over a water bath until it ceases to lose weight. The whole is then carefully weighed, and after deducting the original weight of the filter and watch glass from the total weight, the remainder represents the quantity of albumin in 50 c.c. of urine, and if this quantity be 0·3 gramme, and the amount of the twenty-four hours urine 1200 c.c., then $\frac{1200 \times \cdot 3}{50} = 7{\cdot}2$ grammes of albumin passed in the twenty-four hours.

5. *Estimation of Lime and Magnesia.*—(*A.*) 100 c.c. of filtered urine are introduced into a glass beaker and solution of ammonia added till a bulky precipitate falls—this is redissolved by the addition of acetic acid (slightly in excess). From this acid solution the lime is precipitated by the addition of a saturated solution of ammonium oxalate. Stand for twelve hours, then collect precipitate (reserve filtrate for *B*) on a small filter, the weight of whose ash is known,[2] and wash it well with the distilled water.

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[1] Vide Example 3.

[2] This is determined by incinerating another filter, of equal weight as the one employed, and weighing its ash.

The filter with precipitate is dried over water-bath at 100° C., then placed in a weighed platinum crucible, and the contents reduced to a white ash. When cool add a few drops of sulphuric acid ; this converts the lime into a sulphate. Again heat to redness this lime, placing lid on crucible to prevent loss by spirting. When cold, weigh. Subtract weight of crucible and ash of filter, the remainder gives quantity of sulphate of lime. And since 3 equivalents of sulphate of lime = 1 equivalent of calcic phosphate (Ca_3PO_4), we multiply the quantity of sulphate of lime obtained by 0·7598 to reckon the lime present in 100 c.c. of urine as phosphate ; or by 0·4118 if we desire to calculate it as caustic lime, CaO. (*B.*) The fluid separated by filtration after the removal of the precipitate caused by ammonium oxalate, and which was reserved, is treated with excess of ammonia. Stand for twelve hours, filter, collect precipitate on small filter whose ash is known, and dry over water-bath 100° C. When dry place filter and contents in a weighed platinum capsule covered with a lid, and the heat gradually raised to almost a white-heat. After being exposed to intense heat for some time, a white shining mass of magnesium phosphate is left at the bottom of the crucible. By weighing and subtracting the weight of the crucible and ash we obtain the amount of magnesium phosphate in 100 c.c. of urine, and by multiplying this by 0·3604, we get the amount of uncombined magnesia.

6. *Estimation of Potash and Soda.*—(*A.*) 50 c.c. of filtered urine, mixed with 50 c.c. of baryta solution (1 vol. saturated solution of barium nitrate, 2 vols. of baryta hydrate solution); stand twelve hours, filter. Of this take 40 c.c. (=20 c.c. of urine), reserving overplus in case of accident. Place the 40 c.c. in a platinum crucible, and evaporate to dryness over water-bath. Incinerate in muffle furnace ; at first gently, but afterwards raising heat to full extent till ash is quite white, covering the mouth of crucible lightly with the lid. When cold, dissolve ash in 50 c.c. of boiling water, and add a few drops of solution of ammonia and ammonium carbonate. Stand for a few hours, filter carefully through *very small* filter. Acidulate filtrate with a few drops of hydrochloric acid, and evaporate to dryness over water-bath in a platinum

capsule whose weight is known. The dried residue in platinum capsule is to be gently heated to drive off ammoniacal salts, then allowed to cool, and the whole weighed. Deducting the weight of the platinum crucible, we have the weight of the potassium and sodium in 20 c.c. of urines reckoned as chlorides. (*B.*) To estimate these separately dissolve the ash in platinum capsule in as little water as possible, and add alcoholic solution of platinum bichloride till the solution acquires a deep yellow colour. Evaporate to near dryness and add 50 c.c. of absolute alcohol and 10 c.c. of ether. Set aside for twenty-four hours, frequently stirring. Filter through weighed filter; wash the precipitate (potassium platino-chloride) with alcohol; dry it, and filter over water-bath 100° C. Weigh, deduct weight of filter: result gives weight of potassium platino-chloride. (Every 100 parts of potassium platinum chloride = 30·51 parts of potassium chloride.) By subtracting the potassium chloride from the total sum of potassium and sodium chloride obtained in the first part of the operation (*A*), the difference gives the quantity of sodium chloride. Now if we multiply the potassium chloride by 0·6317 and the sodium chloride by 0·5302, we get the amounts of potash and soda respectively in 20 c.c. of urine, from which it is easy to calculate the quantity passed in twenty-four hours.

(64) Volumetric Analysis. The standard measure used in volumetric analysis is the cubic centimetre, this being the volume of one gramme of distilled water at 4° C. Instruments for measuring liquids are graduated in cubic centimetres, their multiples and divisions. The instruments employed are:—*Pipettes* (Fig. 23) are small spindle-shaped tubular vessels, plain or graduated so as to contain specific quantities of fluid 10, 20, 50 and 100 c.c. Some are made with a bulb and have a mark on the neck; others are simply cylinders graduated throughout their entire length in c.c.'s.

To use the pipette; the liquid is drawn up by suction till it rises above the mark on the neck, the moistened forefinger being then pressed over the mouth, and the pipette withdrawn from the fluid: any fluid adhering to the outside of the instrument must be removed. The forefinger is then partially raised and the liquid allowed to sink down to the mark, and when this point is reached the outflow is arrested by replacing the finger; the liquid can then be conveyed where it is required.

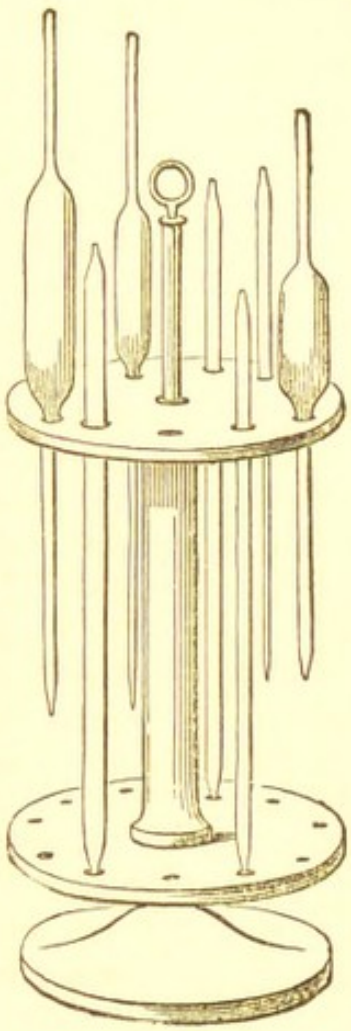

FIG. 23.

Beakers are small glass vessels useful for holding the fluids into which the test liquids are dropped.

They are of various sizes, from 8 to 12 ozs. capacity being most convenient; their usual form is represented at *d*, Fig. 24.

Burettes are graduated tubes of different forms, the most useful is Mohr's, and that only will be described.

It consists of a straight tube about 18 inches in length graduated with a scale, 0° being at the top and 50° at the bottom (Fig. 23). The tube is open at the top, but at the bottom it is drawn out and contracted and fitted into a piece of caoutchouc piping at the

end of which is a fine glass jet. To prevent the liquid running out, the caoutchouc piping is compressed with a pinch-cock. The burette is charged by pouring in the fluid at the top and filling it quite full ; the pinch-cock is then pressed and a few drops allowed to escape till the *under border of the dark zone* of the fluid corresponds with 0° of the graduated scale, Fig. 24. This dark border can be better observed if a sheet of white paper be placed behind the burette.

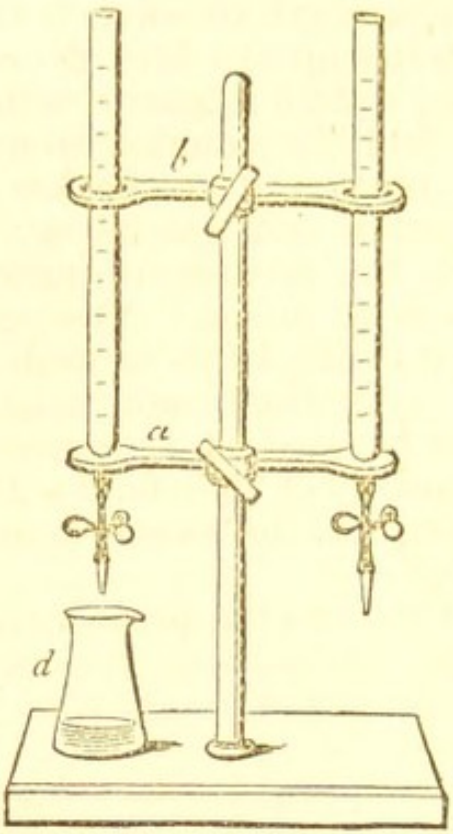

FIG. 24.

The burette being charged, and the fluid for examination measured off by the pipette into a beaker, we proceed to make the experiment as follows. By pressing the pinch-cock a few drops of the standard solution are allowed to run from the burette into the fluid in the beaker and the mixture is well stirred, and so on. The standard solution is cautiously added till some decided reaction is produced, such as change of colour, a precipitate, or reaction with test-paper. When the process is complete the number of c.c.'s required to effect the reaction is read off and the estimation made as in the following examples :—

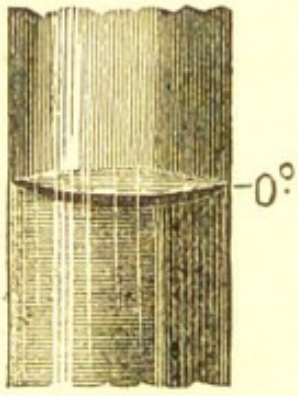

FIG. 25.

Ex. 1. *Estimation of Free Acid in Urine.*—The solution required consists of sodium hydrate standardised, so that 1 c.c. corresponds to 0·01 grm. of crystallised oxalic acid. To make a solution of this strength dissolve 6·35 grms. of caustic soda in distilled water up to 1 litre. Now place in a beaker 100 c.c. of urine, and fill a burette with the standard solution of soda. Add the solution from the burette drop by drop to the contents of the beaker. After each addition shake the mixture and test its neutrality with litmus-paper. When the mixture is quite neutral no more soda solution is to be added. Now as every c.c. of the soda solution used to effect this neutralisation corresponds to ·01 grm. of crystallised oxalic acid, therefore if 3 c.c. of soda solution be used to neutralise 100 c.c. of urine, the degree of acidity of that fluid will be ·03; and from this the acidity of the twenty-four hours' urine can be easily calculated.

Ex. 2. *Estimation of Urea.*—This can be performed by two methods viz., Liebig's and Russell and West's. The latter is considered most convenient for ward work, though the former is more accurate.

(A.) *Liebig's Method.*—This is based on the fact that when a solution of mercuric nitrate is added to a solution of urea an insoluble compound of urea and mercury is formed. If we continue to add the mercuric solution as long as the precipitate is formed, a point is reached, when, on addition of sodium carbonate, a yellow colour is produced by the appearance of hydrated oxide of mercury. This indicates that all the urea has been precipitated by the mercuric solution. The standard solutions required for the process are—

(*a*) The solution of mercuric nitrate, made by dissolving 77·2 grms. of pure, dry mercuric nitrate in strong nitric acid, evaporating the solution to the consistence of syrup, and diluting up to 1 litre with distilled water. 1 c.c. of this solution corresponds to 0·01 grm. of urea.

(*b*) A saturated solution of barium hydrate (2 vols.) and nitrate (1 vol.).

(*c*) And a concentrated solution of sodium carbonate.

Process.—The urine is collected for twenty-four hours

and carefully measured ;[1] of this urine measure off with a pipette 20 c.c. and precipitate the phosphates and sulphates by the addition of 20 c.c. of a saturated solution of barium nitrate and hydrate, and add a few drops of solution of silver nitrate to precipitate the chlorides, and allow the precipitate to subside to the bottom of the vessel containing the urine. Filter, and take of the clear filtered solution 20 c.c., which contains 10 c.c. of urine. Now fill a Mohr's burette with 50 c.c. of the standard solution of "mercuric nitrate," and allow it to fall gradually into the urine. Add first of all 5 c.c. of mercuric solution, stirring well with a glass rod, then remove a drop of the mixture and let it fall on a piece of paper saturated with sodium carbonate ; if no yellow stain is given, add 5 c.c. more of the mercuric solution, and again test ; if there is no result add 1 c.c. of the solution at a time till the yellow colour appears on the test paper ; when this is the case the process is completed, and the estimation can be made. Thus since each c.c. of the mercuric solution precipitates ·01 grm. of urea, therefore the number of c.c.'s of the mercuric solution used will denote the quantity of urea in 10 c.c. of urine ; and if the total quantity of urine passed in twenty-four hours be multiplied by the number of c.c.'s of mercuric solution used, and divided by ten, the quantity of urine submitted to analysis, the amount of urea eliminated in the twenty-four hours will be obtained ; for example, 1520 c.c. of urine are passed in twenty-four hours, 10 c.c. are used for analysis, and 24 c.c. of mercuric solution are employed to precipitate it ; then, $\frac{1520 \times \cdot 24}{10} = 36 \cdot 48$ grms. of urea.

(B.) *Russell and West's Method* is based on the fact that hypobromous acid decomposes urea into water, carbonic acid, and nitrogen. The latter gas is collected alone in a graduated tube. The process is as follows : a flask of about 100 c.c. capacity is fitted with tightly-fitting perforated cork, and attached by means of india-rubber tubing ; and a short glass tube is attached to a graduated tube filled with water, which is turned upside

[1] If albumin is present in the urine, it must be coagulated and removed by filtration.

down in a tall cylinder also filled with water. Place in the flask 25 c.c. of hypobromide of soda (100 grms. of sodium hydrate dissolved in 250 c.c. of water, and the cold solution mixed with 25 c.c. of bromine), at the same time place in the flask a small test-tube containing 5 c.c. of urine, taking care that the contents of the test-tube do not as yet mix with the hypobromide solution. Now attach the flask to the graduated cylinder, and plunge it in water of the same temperature as that in the cylinder.[1] Now tilt the flask so that the urine may freely mix with the hypobromide. The reaction now begins and the gas depresses the water in the graduated tube; in about fifteen minutes the process is complete, and the amount of gas standing in the graduated tube can be read off. If the tube is graduated so that each measure represents one gramme of urea in 100 c.c. of urine, then to calculate the quantity in the twenty-four hours is only a matter of proportion.

3. *Estimation of the Alkaline and Earthy Phosphates.*—An acid solution of uranic nitrate added to a solution of phosphoric acid is decomposed, and a precipitate of uranic phosphate is thrown down. Uranic nitrate gives a reddish stain when dropped in ferrocyanide of potassium test-paper, but uranic phosphate does not give a coloration. As long, therefore, as the uranic phosphate is precipitated on the addition of uranic nitrate no brown stain will be given to a ferrocyanide of potassium test-paper, but as soon as a precipitate ceases to be formed the coloration is given, because then free uranic nitrate appears in the mixture. It is on this fact that the process for estimating phosphoric acid by means of uranic nitrate is based. It requires the following solutions and test-paper :—

---

[1] In making a series of observations in a given case, it is of importance that the temperature of the water should always be the same—especially that surrounding the flask. An increase or decrease of temperature makes a considerable difference in the gas volume; the temperature therefore of the water employed should always be constant. Moreover, to ensure entire accuracy correction should be made for daily variations of barometric pressure.

(*a*) Uranic nitrate solution (1 c.c. = 005 of anhydrous phosphoric acid) is made by placing 35·495 grms. of uranic nitrate in a litre flask, and filling up with distilled water to the mark on the neck of the flask.

(*b*) Sodium acetate solution, to be added to the mixture containing the phosphoric acid, in order to insure the precipitation of uranic phosphate of constant composition. To 100 grms. of sodium acetate add 100 c.c. of strong acetic acid, dilute up to one litre with distilled water.

(*c*) Ferrocyanide of potassium test-paper.—Dissolve a few grains of ferrocyanide of potassium in a drachm of water. Place some ordinary filter-paper on a white plate, and moisten it with the solution thus made.

Process.—Collect the urine passed during the twenty-four hours, and carefully measure and filter; of this urine measure off by means of a pipette, 50 c.c. into a small beaker, and add 5 c.c. of saturated sodium super-acetate solution and heat the mixture in a water-bath to 90° or 100° C. Then add from a Mohr's burette, a few drops at a time, the standard solution of uranium nitrate, and after each addition stir the mixture with a stirring-rod, and transfer a drop to the ferrocyanide of potassium test-paper. Continue the addition of the uranic nitrate till a faint brown stain appears on the test-paper. As 1 c.c. of the uranic nitrate solution corresponds to ·005 grm. of phosphoric acid, therefore, if 17 c.c. of uranium nitrate solution be required to produce the brown stain, then 17 × ·005 = ·085 grm. of phosphoric acid in the 50 c.c. of urine taken for analysis; and if the patient passed 1250 c.c. of urine in the twenty-four hours, then that quantity multiplied by ·085 grm. the amount of phosphoric acid precipitated from 50 c.c. of urine, and divided by 50 c.c., the quantity of urine used for analysis, will give $\frac{1250 \times ·085}{50} = 2·12$ grms. of phosphoric acid in the twenty-four hours urine in combination with the earthy and alkaline bases.

To determine the alkaline and earthy phosphates

separately, proceed as follows. Having determined the total phosphoric acid by the above process, the earthy phosphates must be removed, and their amount determined quantitatively ; this deducted from the total phosphoric acid gives the amount of the phosphoric acid in combination with alkaline bases.

Determination of the Phosphoric Acid combined with the earths.—Take 50 c.c. of filtered urine, and add liq. ammonia till a distinct alkaline reaction is produced ; then set aside for twelve hours. Filter. Wash the precipitate, which consists of earthy phosphates, with dilute liq. ammonia. Then dissolve the precipitate in the smallest possible quantity of acetic acid (2 c.c. to 3 c c.) and add 5 c.c. of the sodium acetate solution, heat the filter and wash with distilled water to bring volume up to 50 c.c. Heat the mixture in water-bath, and then add from a Mohr's burette till a brown stain is given to ferrocyanide of potassium test-paper. Now to precipitate the total phosphoric acid 17 c.c. of uranic nitrate, where required ; on this occasion it will be found that less has been used —say 7 c.c. ; then as 1 c.c. uranic nitrate solution equals ·005 grm. of phosphoric acid 7, × ·005 = ·035 grm. of phosphoric acid in combination with the earthy bases. And as the patient passed 1250 c.c. of urine in the twenty-four hours, then $\frac{1250 \times \cdot 035}{50} = \cdot 87$ grm. of phosphoric acid is in combination with the earthy bases in the twenty-four hours' urine. Now deduct this from the total quantity of phosphoric acid passed in the twenty-four hours, and the remainder represents the *phosphoric acid in combination with the alkaline bases;* thus :

Total phosphoric acid in 24 hours	2·12
Phosphoric acid combined with earths, ditto .	·87
Phosphoric acid combined with alkaline bases.	1·25

4. *Estimation of Chlorine.*—A. Liebig's Method.—When a weak solution of sodium chloride is mixed with a weak solution of mercuric nitrate both salts are decomposed ; nitrate of soda and mercuric chloride being formed, both however remain in solution and are not precipitated. This fact has been made use of for the

determination of chlorine in urine. A dilute solution of mercuric nitrate is dropped into urine, and when the fluids come in contact a white precipitate forms which disappears on shaking. This precipitate is a combination of urea and mercuric oxide (§ 35, Test 4). Sodium chloride being present in the mixture, the mercuric nitrate is converted into mercuric chloride, which does not throw down urea in a weak acid solution. When however all the sodium chloride is exhausted the mercuric nitrate will no longer be changed into mercuric chloride, but will combine with the urea and a permanent precipitate will be formed. When this occurs the process is complete. The solutions required are :—

(*a*) Mercuric nitrate solution (1 c.c. = ·01 chloride of sodium) contains 17·06 grms. of mercury in 1 litre.

(*b*) Barytic solution, for precipitating the phosphates and sulphates from urine, contains 1 vol. saturated solution barium nitrate to 2 vols. barium hydrate.

Process.—Mix 40 c.c. of urine with 20 c.c. of the barytic solution. Shake the mixture well and filter it through a dry filter. Measure off 15 c.c. of the clear liquor into a mixing jar. If it is alkaline to test-paper, it must be neutralized with nitric acid, so as to have the slightest possible excess of that acid. If the filtered solution is found to be acid, before adding the nitric acid, the quantity of barytes added to the urine was, perhaps, too little. To test this, add to a little of the filtered non-acidified liquor, a few drops of the barytic solution. If this produces a precipitate, you must begin afresh :—Mix 20 c.c. of urine with 20 c.c. of the barytic liquor, and measure off 20 c.c. of the filtered mixture for analysis. The 15 c.c. of the mixture contains 10 c.c. of urine separated sulphates and phosphates. To this mixture, the mercuric solution is to be run gradually from a Mohr's burette, until, after well stirring and shaking the mixture in the jar, there appears a *permanent* precipitate. This precipitate is produced by the action of the mercury on the urea in the urine, after the action of the salt on the mercury is concluded. The operation is then finished. The number of c.c. of mercuric solution used, as shown by the scale on the burette,

indicate so many times 10 milligrammes (0·010) of chloride of sodium contained in 10 c.c. of the urine.

B. Nitrate of Silver Method.—When nitrate of silver is dropped into a neutral solution of sodium chloride and neutral potassium chromate, the chlorine is thrown down in the form of chloride of silver, when the whole of the chlorine is thrown down; then, when the silver solution is added it is converted into chromate of silver which gives the mixture a permanent red colour. When this occurs the process is complete. The solutions required are:

(*a*) Nitrate of silver solution (1 c.c. = ·01 grm. sodium chloride, or ·006 grm. of hydrochloric acid) 20·063 grms. of pure fused nitrate of silver are dissolved in distilled water to fill 1 litre.

(*b*) Yellow potassium chromate solution; saturated.

Process.—Collect the urine for 24 hours, carefully measure. Filter a portion of this urine and measure off, by means of a pipette, 50 c.c. into a small beaker, and add a few drops of sodium carbonate solution, to render it neutral, and dilute with distilled water up to 100 c.c. A few drops of potassium chromate solution are now added and a few c.c.'s of the standard solution of silver nitrate run into the mixture from a Mohr's burette; agitate. Continue to add a c.c. or so of the standard solution till a red colour appears when the mixture is agitated (red silver chromate). Now since 1 c.c. of the silver nitrate solution is equal to ·006 grm. of hydrochloric acid, therefore if 17 c.c. of silver nitrate be used, the 50 c.c. of urine will contain ·102 grm. of hydrochloric acid, and if 1500 c.c. of urine be passed in the 24 hours, then $\frac{\cdot 102 \times 1500}{50} = 3\cdot02$ grms. of hydrochloric acid.

5. *Estimation of Sulphuric Acid.*—The process consists in adding a solution of chloride of barium to a given quantity of urine so long as a precipitate of barium sulphate is formed. The solution required is:

Barium chloride solution (1 c.c. = ·01 grm. of sulphuric acid). Dissolve 30·5 grms. of dry crystallized barium chloride in distilled water to 1 litre.

Process.—Collect the urine for twenty-four hours, and carefully measure. Of this urine measure off, by means of a pipette, 50 c.c. into a beaker, add 10 drops of hydrochloric acid, and boil. Then from a Mohr's burette allow the standard solution of barium chloride to run into the mixture till a precipitate ceases to be formed. Now as 1 c.c. of the barium chloride solution equals ·01 of sulphuric acid therefore if 10 c.c.'s of this solution are required the quantity of sulphuric acid in 50 c.c. of urine will be 0·1 grm. ; and by multiplying this quantity by the total amount of the twenty-four hours' urine, say 1200 c.c., and dividing by 50 c.c. the quantity of urine employed for analysis ; we have, $\frac{0{\cdot}1 \times 1200}{50} = 2{\cdot}4$ gms. of sulphuric acid passed in the twenty-four hours.

6. *Estimation of Sugar.*—When an alkaline solution of *cupric* sulphate is boiled with a solution of sugar, the salt is reduced and a red precipitate of cuprous oxide is thrown down. This reduction of cupric salts furnishes a method for estimating the amount of sugar in solution. The following solutions are required :

(*a*) The Copper Solution.—Dissolve 34·63 grms. of crystallized cupric sulphate in distilled water, and make up to 1 litre. 1 c.c. of this solution corresponds to 0·005 grm. of sugar.

(*b*) The Alkaline Tartrate Solution — 173 grms. of sodium and potassium tartrate (Rochelle salt) and 80 grms. of potassium hydrate are dissolved in water to the measure of 1 litre. 10 c.c. of this solution are to be used with every 10 c.c. of the cupric solution.

(A.) *Estimation of Sugar in Urine.*—The urine passed during the 24 hours is collected and carefully measured. Of this urine measure off 10 c.c. with a pipette, and dilute with distilled water to 200 c.c. and fill a burette with a portion of diluted urine.

Into a porcelain basin containing 50 c.c. of distilled water, measure off 10 c.c. of standard copper solution and 10 c.c. of alkaline tartrate solution, and boil the mixture.

When the alkaline copper solution has reached the

boiling point a few drops of the dilute urine are run into it from the burette; at first the addition only makes the copper solution turbid with a greenish-red precipitate, which subsequently on the further addition of urine acquires a deeper red and settles readily at the bottom of the porcelain vessel. After each addition of urine the precipitate should be allowed to settle and the vessel slightly tilted so as to observe the colour of the supernatant fluid; when this becomes perfectly colourless the process is complete and the estimation can be made as follows: Suppose the patient passes 4110 c.c. of urine in the 24 hours and 30 c.c. of dilute urine are required to reduce the copper solution, then 4110 c.c. ÷ 30 gives the quantity of sugar in the 24 hours' urine.

(B.) *Quantitative Estimation of Sugar in Milk.*—Curdle say 100 c.c. of fresh milk and remove the curds by filtration. Dilute 10 c.c. of the filtrate with distilled water to the volume of 200 c.c. and with this fill a Mohr's burette; for the remainder of the process proceed as directed in the quantitative estimation of sugar in urine.

(C.) *Roberts's Fermentation Process.*—The urine is collected for 24 hours and carefully measured, and 4 ounces of this taken and placed in an 8-ounce bottle together with a small piece of yeast, and in another bottle a similar quantity of urine but no yeast. The two bottles are now to be put aside in a warm place for 24 hours, and the contents of each having been poured into two urine glasses their respective specific gravities are to be taken. *The difference of each degree lost in the urine which has the yeast indicates the presence of one grain of sugar in every fluid ounce of urine.* For example, a patient passes 160 ounces of urine in the 24 hours; and the specific gravity of the urine in the bottle without the yeast is 1·042, and in the bottle with yeast 1·033, or 9 degrees less (which represents the loss occasioned by the formation of carbonic acid), and each degree thus lost represents one grain of sugar; then 160 ounces multiplied by 9 gives 1440 grains of sugar passed in the 24 hours.

7. *Estimation of Iron.*—A definite quantity of the ash must be dissolved in a little hydrochloric acid, and heated; to the acid solution some sodium sulphite is added and the mixture boiled till the liquid is colourless,

diluted with distilled water and cooled. A Mohr's burette is then filled with a standard solution of potassium permanganate. This solution of potassium permanganate is made thus: A concentrated solution of 10 parts of potassium hydrate is added to 8 parts of manganese peroxide and 7 parts of potassium chlorate, the mixture evaporated to dryness, and the residue heated in a platinum crucible to redness, until the potassium chlorate is decomposed. Triturate the green mass, and boil until the green colour has changed to the violet tint of the permanganate; decant the solution, remove the precipitate, and filter through asbestos. To determine the strength of the solution, weigh off 7·543 grms. of pure, dry, crystallized potassium ferrocyanide, corresponding with 1 grm. of iron, and dissolve in distilled water, added to fill a litre measure. 10 c.c. of this solution represents 0·010 grm. of iron. Now take 10 c.c. of the potassium ferrocyanide solution, and dilute with 50 c.c. of distilled water acidulated with a little hydrochloric acid, place the vessel containing the solution on a sheet of white paper, and add the permanganate solution till a yellowish-red colour appears in the fluid on agitation. If 20 c.c. of the permanganate solution is used, the graduation is complete, and 1 c.c. of it will correspond to 0·0005 grm. of iron. But if more or less than 20 c.c. are required, then the permanganate solution must be concentrated or diluted till the required strength, viz. 20 c.c., to produce the yellow-red colour with 10 c.c. of potassium ferrocyanide solution, is attained. This is added in small quantities to the colourless solution of iron, agitating after every addition the vessel that contains the mixture. When the operation is completed the iron solution acquires a pale rose red which does not disappear on agitation. The number of c.c.'s of the standard solution used from the burette must now be taken; and as 1 c.c. of the permanganate solution corresponds to ·0005 grm. of iron, then if 5 c.c. of the standard solution be used, the quantity of iron contained in the ash will be ·0025 grm.

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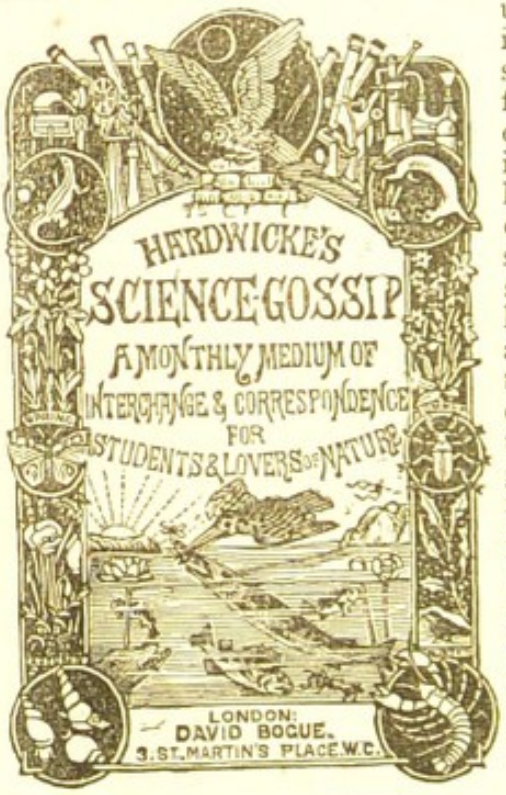

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