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by J. Dixon Mann ... With illustrations.**

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PHYSIOLOGY AND PATHOLOGY

OF THE
URINE

WITH METHODS FOR ITS EXAMINATION

BY

J. DIXON MANN, M.D., F.R.C.P.

PHYSICIAN TO THE SALFORD ROYAL HOSPITAL; PROFESSOR OF FORENSIC
MEDICINE IN THE VICTORIA UNIVERSITY OF MANCHESTER

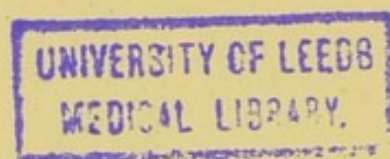
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PHYSICIAN TO ST. MARY'S HOSPITAL, MANCHESTER;

CONSULTING OBSTETRIC PHYSICIAN TO THE MANCHESTER ROYAL
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IN REMEMBRANCE OF OUR LONG FRIENDSHIP AND IN

GRATEFUL RECOGNITION OF MANY KINDNESSES

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PREFACE

THIS volume is intended to serve as a clinical guide in the diagnosis and treatment of disease. Descriptions are given of the constituents of the urine, its physical properties (which have recently received much attention), and its chemical reactions, along with the methods to be followed in its examination; these are severally dealt with proportionally, in the judgment of the author, to their importance in relation to clinical medicine. From the physiological and pathological standpoints, some of the urinary constituents are closely associated, and, for convenience of discussion and of reference, have been grouped together irrespective of their chemical constitution. The systemic conditions are described under which each urinary component occurs in excessive or defective amount; and some of the more important diseases and pathological deviations, which are attended by distinctive changes in the urine, are separately considered in relation to the effects produced by them on its composition and physical characteristics.

As being conducive to a due appreciation of the significance of the changes which take place in the urinary constituents, concise accounts are given of the results of the most recent investigations in metabolism (so far as it affects the urine) as well in the normal state as when modified by disease; references being added by means of which the original papers may be consulted.

The distinction between chemical processes which pertain to the clinical laboratory and those which are more suitable to the laboratory for pathological chemistry is indefinite, and to

a large extent is determined by the skill and inclination of the investigator; therefore, whilst strictly clinical methods—such as those which are adopted for the detection and estimation of albumin, sugar, urea, pigmentary bodies, and the like—are allotted the chief place, the interests of investigators who desire to carry their researches further have not been overlooked. The obvious result is that, although this is avowedly a clinical guide, many processes are described in it which are beyond the scope of the clinical laboratory.

The foundations laid by pathological chemistry are ever changing: what is accepted to day may to-morrow be merely as a tale that is told. Hence, the author has endeavoured to place before the reader the latest survey of those branches of biological chemistry with which this volume deals.

January 1904.

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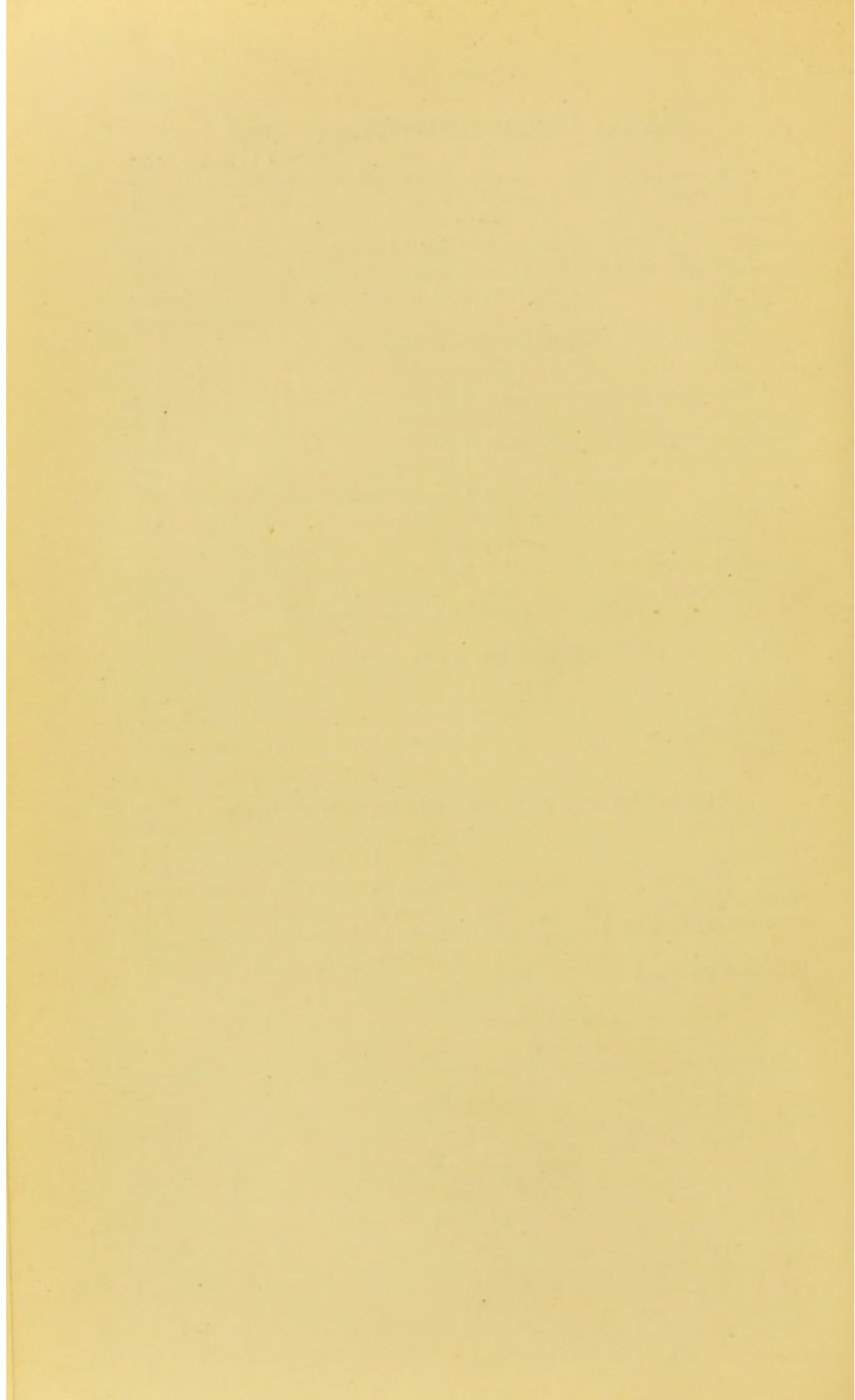
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THE GENERAL CHARACTERISTICS OF URINE.

URINE is a complex liquid in which a number of the end products of metabolism, along with inorganic substances, are held in solution. Considerable variations both in the amount and in the composition of the urine are consistent with the healthy condition; for the most part, such variations in composition relate to increased or diminished percentage of the normal constituents of urine, although some abnormal substances may occasionally be present without the occurrence of any recognisable deviation from health. The constituents of normal urine may be classified according to their acid or their basic properties, each class being partly inorganic and partly organic. The inorganic acids comprise: hydrochloric, sulphuric, phosphoric, and carbonic; the bases are potassium, sodium, ammonium, calcium, and magnesium, with traces of iron. The organic acids are represented by uric, oxalic, hippuric acids, along with traces of some of the volatile fatty acids—acetic, formic, propionic, and butyric—and of some oxyacids of the aromatic group; the urinary pigments may be said to belong to the acid group. The organic bases comprise urea, creatinin, the xanthin bases, and others which only appear in traces.

The **quantity** of urine which is compatible with a healthy condition is susceptible of wide variations, due partly to the amount of fluid that is imbibed, and partly to the activity of the sweat glands; forty to fifty ounces may be regarded as the usual daily amount for an adult, representing about two-thirds of the liquid swallowed. In hot weather, when the skin-glands are very active, the proportion of urine to liquids swallowed is much less; and conversely, the lessened perspiration caused by a low temperature increases the amount of urine relatively to that of the liquids imbibed. The quantity of the urine secreted during the day is greater than in the night, the relation being 100:50 or 60; or occasionally 100:80.

Quinke¹ observed, in patients suffering from certain diseases, that the day urine may be exceeded by the night urine in amount; instead of being 100:50 or 60, it may be 100:100 or 200. This nocturnal polyuria is not due to simple increase of water, there is at the same time an increase in the solid urinary constituents. It occurs in heart and kidney disease, in elderly people with arteriosclerosis, in prostatic hypertrophy, and in diabetes insipidus. Laspeyres² has also observed in disease of the cardiac valves and musculature, in renal disease, in some vesical diseases, and occasionally in diabetes, that the normal relation may be inverted to the extent of 100:385. He attributes the nocturnal increase to the numerous claims on the circulation during the day, even when the patient remains in bed; under the recuperating influence of more complete repose, the tonus of the heart-muscle and of the vascular walls improves, with the result that some of the liquids which were retained during the day are excreted in the night.

The pathological conditions in which the quantity of the urine excreted in the twenty-four hours is increased are: Diabetes insipidus and mellitus, contracting and lardaceous kidney, at the crisis of some fevers, in various nervous affections—especially in hysteria and epilepsy—and also in some injuries to the brain. In some of these conditions the increase is due to the amount of liquids imbibed; in others, of a more temporary nature, to the rapid rise of the blood-pressure. The amount of urine passed may considerably exceed the volume of liquid imbibed, as when absorption occurs of fluids which have accumulated in the cavities of the body or under the skin. In diabetes insipidus enormous quantities of urine may be passed, even up to 800 ounces daily, and for a time the daily amount of urine may exceed the amount of liquid that is drunk; as soon as the tissues have parted with all their spare fluids the excess comes to an end. When the proportion borne by the urine to the imbibed liquids is estimated, allowance must be made for the watery constituents of the solid food that is eaten.

The secretion of urine may be diminished in the acute stage of Bright's disease, in heart disease when the blood pressure is low, in fevers and febrile conditions, in gastric catarrh attended by vomiting, in diarrhoea, in cases where accumulation of fluid is taking place in the cavities of the body or under the skin, and in suppression of urine, or when there is a mechanical obstruction to its flow.

The colour of healthy urine varies in accordance with its concentration; it is therefore darker in summer than in winter, and

¹ *Arch. f. exp. Pathol.*, 1893.

² *Deutsch. Arch. f. klin. Med.*, 1900.

after rising in the morning than during the rest of the day; the usual colour is amber-yellow. In health, light coloured urine occurs after the ingestion of large quantities of liquid; in disease, it occurs in diabetes insipidus, in acute diabetes mellitus, in hysteria and allied conditions, and in granular kidney. An unusually intense yellow colour is suggestive of the presence of bile-pigment or of excess of urobilin, or of urochrome; the two substances first named may produce a much darker colour, like that of strong ale; when much bile-pigment is present the urine may be black, like porter.

In fevers and febrile diseases, such as acute rheumatism and pneumonia, the urine is usually deep brown in colour, partly due to concentration and partly to the presence of excess of urobilin. Red-tinted urine suggests the admixture of blood; if the amount of blood be small, and if it be derived from the kidneys, the urine will have a smoky appearance. When free hæmoglobin liberated from the blood corpuscles is present, the urine in bulk may appear quite black; in smaller volume, or when diluted with water, it is red. The presence of some of the derivatives of blood may alter the colour of the urine; a burgundy-red tint suggests the presence of hæmatoporphyrin, although the tint is really caused by some other unknown pigment which may accompany hæmatoporphyrin, especially after the prolonged administration of sulphonal or trional. The tint of the urine may be changed by the presence of various adventitious colouring-matters: rhubarb gives it a rich golden yellow; phenol and its derivatives a green or dark brown colour. In some morbid conditions the colour materially changes if the urine, after being passed, be allowed to stand exposed to the air; this occurs in carbouluria, alkaptonuria, and melanuria.

In the healthy state the urine is always limpid when passed, and it remains so after many hours' standing. A faint cloud of mucus, in which are entangled a few epithelial cells, slowly subsides; this is known as the *nebecula*, and is best seen when the urine has been allowed to stand in a tall glass vessel. Occasionally the *nebecula* may be seen floating midway in the column of urine; this is sometimes due to excess of urochrome, or to high specific gravity of the urine, and sometimes to the inclusion of small air bubbles in the cloud, and to its being more than usually diffuse in character. When occupying its usual position, the *nebecula* is subject to considerable variations in density and translucency without affording any indications of disease. The presence of vulval and vaginal epithelium and mucus tends to make the *nebecula* denser in women than in men; the leucorrhœa, to which some women are subject without obvious interference with health, may impart to it a semi purulent

appearance. On the other hand, when abnormally large quantities of urine are voided—as in diabetes mellitus and insipidus, and in hysterical polyuria—the amount of mucus and epithelium present in the few ounces of urine contained by the urine-glass is often insufficient to yield any visible deposit.

In many pathological conditions the cloud is much heavier, on account of the presence of renal elements, as occurs in Bright's disease; pus or muco-pus causes the deposit to be still heavier and more opaque. In the subacute and chronic stages of gonorrhœa, and in gleet, the urine contains whitish filaments and specks which are composed of consolidated collections of pus and epithelium cells from the lacunæ of the urethra. The turbidity of urine that is turbid when voided may be due to pus, to earthy phosphates, or to bacteria; on rare occasions it is due to finely divided fat. Turbidity that only appears after the urine has stood for a short time is due to amorphous urates.

After a varying interval urine shows the results of decomposition. In some instances, what has been termed "acid fermentation" occurs, manifested by the formation of crystals of uric acid, of acid urate of ammonium, and of calcium oxalate, along with amorphous urates. Subsequently, and more commonly without the appearances of the acid fermentation, alkaline fermentation sets in, the urine becomes cloudy, and gives off the odour of ammonia due to hydrolysis of the urea by the *Micrococcus ureæ* and other micro-organisms, into ammonium carbonate and carbon dioxide. Exceptionally, a specimen of urine remains free from decomposition for an indefinite period. Probably the protective influence is not always the same; but in some instances phenol compounds are present in excess and they possibly keep the urine sterile. After a time, such urines, whilst remaining perfectly clear and free from the odour of decomposition, will slightly darken in colour owing to the formation of oxidation products of phenol.

The odour of healthy urine is characteristic; that of concentrated urine may be very intense and objectionable even to the patient, whilst in polyuria it is scarcely perceptible. When urine, either in or out of the bladder, is undergoing decomposition it yields an ammoniacal odour; with more advanced decomposition the odour may be putrescent. The presence of acetone may impart the odour of that substance to the urine. Adventitious odours may be produced by the administration of certain drugs and articles of food: turpentine produces a violet-like odour; some drugs, such as copaiba and peppermint, impart their intrinsic odours to the urine; asparagus and garlic cause it to have a very offensive odour.

The **specific gravity** of urine. For clinical purposes this is ascertained by means of an instrument called a urinometer. As usually constructed the instrument is defective; the scale is limited to one side of the stem and is too contracted, rendering an accurate reading difficult; the difficulty is increased by the cylindrical form of the instrument which causes it to rotate when plunged into the urine. A better form of urinometer is made with a flattened body and stem, and a wider scale, which is graduated on both sides of the stem.¹ To take the specific gravity, a cylindrical urine-glass is filled to within an inch from the top with urine that has cooled to the temperature of the surrounding air, the urine being gently poured into the glass so as to avoid the formation of froth; the urinometer is then plunged into the urine, and the division on the scale which corresponds to the surface-level is read off; this represents the specific gravity of urine in relation to that of pure water. The division on a level with the surface of the urine is taken, and not that at the summit of the curve formed by the urine a little way up the stem of the urinometer. To make sure of the reading, it is well to depress the instrument below its floating-point and to take a second reading after it has resumed its position. If greater accuracy is required than the urinometer can afford, a Westphal's specific gravity balance should be used. When the temperature of the urine is materially above 60° F., one degree should be added to the reading obtained for every 8° F. above 60° .

In health the specific gravity of urine usually ranges between 1012 and 1020; if large amounts of liquid are drunk, the gravity may be as low as 1005; in hot weather when the urine is concentrated it may reach 1030 or more. In some diseases the gravity may be very low; in diabetes insipidus it may not exceed 1002, or even 1001; in contracting kidney, also, the gravity is low. Apart from acute diabetes mellitus, the lighter the colour of the urine the lower is its specific gravity; in other words, the specific gravity of urine increases with its concentration, chiefly on account of the large proportion of urea that is present. Diabetic urine is usually very light coloured, but on account of the sugar that it holds in solution its specific gravity is high, reaching 1040 or higher. Albuminous urine may also have a high specific gravity, and the gravity has been found high in chorea (Cox). A rough estimate of the amount of solid matter held in solution in the urine may be made by multiplying the number of degrees of difference in specific gravity, between the urine and that of water, by 2.3, which gives the weight of solids in every 1000 parts. Thus, if the specific gravity of the urine

¹ May be obtained from Mottershead & Co., Manchester.

is 1025, then $25 \times 2.3 = 57.5$; that is to say, that every litre of the urine contains about 57.5 grms. of solid matter. By multiplying the number of grammes of solid matter, obtained as above, by the number of cubic centimetres of urine voided in the twenty-four hours and dividing the product by 1000, the total amount of solids contained in the twenty-four hours' urine is obtained. Inasmuch, however, as solutions containing the same percentages of the various urinary constituents have widely differing specific gravities, the estimation of solids by calculations based on the specific gravity of the urine is far from being accurate.

Reaction.—Healthy urine yields an acid reaction with litmus paper; this reaction is not due to free acid, but to the presence in certain proportions of the monohydric and the dihydric sodium phosphates, the reaction of the former being alkaline and that of the latter acid. Under ordinary conditions the proportion of the dihydric to the monohydric salt is about as six is to four; this determines the acid reaction of urine. The relative proportion of the two salts is subject to considerable variation, which is determined by various causes. The character and the amount of the food that is eaten exercise a considerable influence: animal food, being poor in bases, increases, whilst vegetable food which is rich in bases, diminishes the acidity of the urine. When introduced into the organism, the bases which are contained in food, especially the combinations of the alkalies with the organic acids such as exist in fruit and vegetables, are quickly converted into carbonates, and by combining with the acid products keep the acidity of the urine in check. If from any cause the food-bases are insufficient to prevent excessive acidity, some of the ammonia which is split off from the tissue-proteids takes their place and neutralises the excess.

After a meal the urine becomes less acid, and for a short time may occasionally have an alkaline reaction. This is due to the secretion of hydrochloric acid into the stomach, so that the bases with which it was combined in the blood are set free; these, along with the salts contained in the food, react on the urinary phosphates in such a way as to increase the proportion of monohydric phosphates, and consequently to diminish the acidity of the urine. If the secretion of hydrochloric acid is arrested, this variation in the reaction of the urine does not occur. Hofmann¹ states that in a patient who had undergone total extirpation of the stomach, the acidity of the urine was not diminished after food. The "alkaline tide," as it is called, is only present while the peptic digestion is at

¹ *Münchener med. Wochenschr.*, 1898.

its greatest activity. To obtain evidence of its presence, the bladder must be emptied at frequent intervals after the meal, the reaction of each sample being taken separately. In a short time the alkaline tide subsides and the urine again yields its usual acid reaction; inasmuch as the volume of the acid urine greatly exceeds that which is alkaline, or which is approaching alkalinity, the reaction of the aggregate twenty-four hours' urine is acid.

The acidity of urine is increased in febrile conditions, in diabetes, dyspepsia, leucocythæmia, scurvy, pernicious anæmia, and in pregnancy. Prolonged exercise, by which tissue-changes are promoted, with consequent liberation of acid products, intensifies the acidity of the urine. Concentrated urines are usually more acid than urines which are dilute. After being voided, the acidity of urine may be increased owing to the occurrence of "acid fermentation," a condition, however, which is exceptional, and is of short duration.

Urine may become **alkaline** in two distinct ways: (*a*) from combinations of the fixed alkalies, or of the alkaline earths, such as the alkaline carbonates, or the dibasic, or the earthy phosphates; and (*b*) from the presence of the volatile alkali in the form of ammonium carbonate. When the alkalinity of urine is due to (*a*), the urine is secreted as an alkaline fluid. Alkaline urine of this type may be due to excessive vomiting, especially that which occurs in dilatation of the stomach, or to any other condition that prevents the gastric juice taking its normal direction. Excessive perspiration tends to reduce the acidity of the urine; in anomalous states of debility, in neurasthenia, in phthisis, and in some anæmias the urine may be alkaline. When the alkalinity is due to (*b*), it almost always results from the action of micro-organisms—chiefly the *Micrococcus ureæ*—by which the urea is decomposed and ammonium carbonate is formed; this takes place in the bladder; when the urine leaves the kidneys, it has the normal, acid reaction. All conditions which interfere with the natural and complete emptying of the bladder, such as stricture of the urethra, enlarged prostate, paralysis from myelitis, or other diseases of the cord, along with the introduction into the bladder of unclean catheters, predispose to, and produce ammoniacal urine.

By the administration of the alkalies, their carbonates, or their vegetable-acid salts, such as citrate or acetate of potash, the urine can readily be made to yield an alkaline reaction. Citrates and acetates of the fixed alkalies are converted by the tissues into carbonates which appear in the urine and render it alkaline. Citrate and acetate of ammonia, however, do not render the urine alkaline, as

the ammonium carbonate which is formed is hydrolysed by the liver cells and is transformed into urea before it reaches the kidneys.

Whilst it is easy to make the urine alkaline by means of saline drugs, it is difficult, when patients habitually secrete alkaline urine, to reverse the action and make it acid; acid medicines have but little power in this direction. Hutchison¹ has obtained good results from frequent half-drachm doses of the acid phosphate of sodium, by which the acidity of the urine is distinctly increased.

The reaction of urine is ascertained by means of litmus-paper; that which has a glazed surface and is coloured on one side only is the best. If a slip of blue litmus-paper is dipped into distinctly acid urine and is held there for a few seconds, its colour changes to red; if the urine is but feebly acid, the blue changes to purple. Red litmus-paper held for a few seconds in alkaline urine is rendered purple by feeble alkalescence, and blue if the alkalescence is more pronounced. When the alkalinity is due to a fixed alkali, the blue coloration that it imparts to red litmus-paper is permanent; when it is due to ammonia, the blue colour disappears on exposure to the air and gives place to the original red. Occasionally the dihydric and the monohydric phosphates are present in urine in such proportions that each salt gives, faintly, its own reaction—the acid salt turns blue litmus-paper purple, and the monohydric salt turns red litmus-paper bluish; this double reaction is known as the amphoteric reaction. Urine is never neutral to litmus paper.

The determination of the degree of acidity of urine is less easy than might be supposed. When the acidity of a liquid is due to the presence of a free acid, the degree of acidity may be accurately determined by ascertaining how much of an alkaline solution of a known strength must be added, in order to neutralise the acid; the amount of alkali which is required indicates the degree of acidity. In urine a more complex condition exists, as its acidity is due to the presence of an acid salt, the degree of acidity being determined by interaction between this salt and its alkaline ally. Many more or less complex methods have been devised to overcome this difficulty; but in avoiding one source of error others are encountered, the result being that the best results are obtained by the ordinary titration method which is resorted to in the case of simple acid liquids, although, on account of the imperfect saturation of the phosphates, the estimation is usually too low. Titration is performed by taking 100 c.c. of the urine (if dark in colour it must be diluted with water), adding a few drops of a solution of phenolphthalein and then decinormal solution of sodium hydrate, from a burette, until the indicator

¹ *Brit. Med. Journ.*, 1903.

becomes distinctly red. The number of cubic centimetres of the decinormal solution necessary to produce the reaction is the measure of the acidity of the urine, and may be expressed in the equivalent proportion of normal hydrochloric acid.

The degree of alkalinity of urine may be estimated by adding a little phenolphthalein to 100 c.c. of the urine, and then adding decinormal sulphuric acid until the red colour due to the indicator is destroyed. Each cubic centimetre of the acid represents 0.0106 gm. of sodium carbonate.

INORGANIC CONSTITUENTS.

THE inorganic constituents of urine comprise certain members of the non-metallic group of elements—*chlorine, sulphur, phosphorus, carbon, nitrogen, silicon, fluorine, and hydrogen*—the combinations of which with oxygen and hydrogen constitute the urinary inorganic acids, together with certain metals—*sodium, potassium, ammonium, calcium, magnesium, and iron*—which form the bases. Urine also contains small amounts of free *oxygen, nitrogen, and carbon dioxide* in the gaseous form.

INORGANIC ACIDS.

HYDROCHLORIC ACID. HCl.

Hydrochloric acid is present in urine in combination with small amounts of potassium, ammonium, and magnesium, but it occurs most abundantly in combination with sodium. About 7 grms. of chlorine, equal to about 15 grms. of sodium chloride, are excreted daily. Berlioz and Lépine¹ and Vitali² state that a small proportion of the urinary chlorine is present in organic combination; but their methods have been shown to be unreliable by Petit and Ferrat,³ by Ville and Moitessier,⁴ and by Meillère.⁵ Recently, Bruno⁶ asserts that he has occasionally found a small amount of organic chlorine to be present; it is generally accepted, however, that the presence of organically combined chlorine in urine has not been proved.

Chlorine is chiefly introduced into the system in food, especially in the form of common salt; the amount of the condiment that is ingested largely influencing the percentage of sodium chloride present in the urine. When much animal food is eaten, the urinary chlorine, along with the urea with which it proportionally varies, is increased; conversely, in states of inanition it is diminished. In

¹ *Arch. de Méd. expérim.*, 1894.

³ *Thérapeut. scientif.*, 1894.

⁵ *Ibid.*

² *Boll. Chim. Farm.*, 1897.

⁴ *Compt. Rend. Soc. Biolog.*, 1901.

⁶ *Riforma Med.*, 1901.

febrile conditions the chlorides are usually retained; in some instances, at certain stages of the fever, an increase in chlorine excretion has been observed. An increase also occurs in rickets and in cirrhosis of the liver. In the later stages of chronic nephritis, both parenchymatous and interstitial, retention of chlorides has been observed especially in, or preceding, the uræmic condition (Hofmann¹). According to Marischler,² in parenchymatous nephritis, the kidneys are quite permeable to sodium chloride, the diminished excretion being due to the kidneys holding back water; diuresis is produced by the simultaneous administration of sodium chloride and water, but not if water be withheld. In the exudative stages of pneumonia and pleurisy, the urinary chlorides are materially reduced in quantity and they may be entirely absent.

As pointed out by Hutchison,³ this is due to a true retention of chlorides in the tissues, the daily amount retained averaging 2 grms. of sodium chloride. The lessened excretion usually continues one or two days after the crisis, and it is succeeded by an excess which is considerably beyond the amount contained in the food that is ingested. Diminution of the urinary chlorides also occurs in fevers, especially in typhus and rheumatic fevers, but it is more constant in pneumonia. Hutchison's investigations show that in pneumonia the exudation, and also the sputum which is rich in chlorides, do not account for more than one-third or one-half of the total retention; but that the organs collectively are rich in chlorides. As Van den Bergh⁴ points out, the chloride retention is probably due to an effort to maintain the balance of osmotic pressure. The molecular concentration of the blood is so much increased as to necessitate an equivalent increase on the part of the tissues so as to enable the necessary interchange to take place; this the chloride retention tends to accomplish. In some chronic conditions—in malignant disease, in chronic gastric catarrh, and in other diseases which are attended with anorexia—the chlorides are diminished chiefly on account of the small quantity of food that is eaten; whilst in general œdema and ascites, the diminution is to some extent due to the abstraction and locking-up in the transudations of a portion of the chlorides that otherwise would be excreted. On the other hand, during the stage of rapid absorption of exudative products, the urinary chlorides tend to increase, as they also do in aggravated polyuria, on account of the excessive lixiviation of the tissues.

¹ *Deutsch. Arch. f. klin. Med.*, 1898.

² *Arch. f. Verdauungskh.*, 1901.

³ *Journ. of Path. and Bacteriol.*, 1898.

⁴ *Nederl. Tijdschr. v. Geneesk.*, 1902.

The presence of chlorides in urine is easily demonstrated by pouring a specimen into a test-tube and adding a few drops of nitric acid so as to prevent interference by the phosphates; on dropping in a little solution of silver nitrate a white precipitate forms, which, when the chlorides are present in normal amount, is thick and curdy; when they are materially diminished the urine merely becomes opalescent or turbid. The precipitate is soluble in ammonia but not in nitric acid. If, when performing this test, a second tube containing some urine from a healthy person is dealt with in the same way, a comparison of the results will roughly indicate any pronounced alteration in the excretion of chlorides.

Estimation.—In determining the amount of chlorides in urine, other substances which may be present, and which also form precipitates with silver nitrate, have to be taken into consideration. Many processes, with numerous modifications, have been devised; that of Neubauer and Salkowski¹ is probably the best. To 10 c.c. of the urine in a platinum basin, 1 grm. of sodium carbonate and 2 grms. of potassium nitrate, both free from chlorine, are added. The mixture is evaporated to dryness at a temperature below the boiling-point, and the residue is then heated over a Bunsen flame until it melts and becomes quite white. When cold it is dissolved in water and is carefully transferred to a porcelain capsule; dilute nitric acid is dropped in until the solution is feebly acid, when it is again made neutral with calcium carbonate. A drop or two of a solution of potassium chromate is added and, from a burette, a standard solution of silver nitrate is run in with constant stirring, until the precipitate acquires a reddish tinge, which is the end reaction. Each cubic centimetre of the silver solution—which contains 4.789 grms. of silver nitrate to the litre—is equal to 0.01 per cent. of chlorine. The percentage may be calculated as sodium chloride by multiplying the percentage of chlorine by 1.648.

In this process the object of adding sodium carbonate before evaporation is to prevent loss of chlorine should any ammonia be present, which, in the absence of the carbonate, would escape as ammonium chloride; the excess of sodium carbonate causes any ammonia to pass off as ammonium carbonate. The potassium nitrate prevents the formation of cyanides and cyanates which otherwise would be precipitated with the silver chloride, as also would certain organic substances that are destroyed by the heat.

¹ *Zeitschr. f. physiol. Chem.*, 1877 and 8.

SULPHUR.

Sulphur may be present in urine in several conditions : (a) *Sulphuric acid* ; (b) sulphuric acid conjugated with aromatic products like phenol and indol, forming *ether-sulphuric acid* ; (c) *neutral or incompletely oxidised sulphur* ; (d) *sulphuretted hydrogen*.

SULPHURIC ACID. H_2SO_4 .

(a) Sulphuric acid, chiefly in combination with potassium and sodium, is present in urine to the daily amount of about 2 to 3 grms. ; one-tenth of this is in the form of ether-sulphuric acid. About 80 per cent. of the ingested sulphur, which is mostly derived from the food-proteids, is oxidised to sulphuric acid, a fairly constant relation being maintained between the excretion of sulphuric acid and of nitrogen— $\text{N} : \text{SO}_3 = 5 : 1$; after severe bodily exercise, for example, the output of sulphuric acid keeps pace with the increase in urea. The amount of sulphuric acid in urine is increased by the administration of free sulphur and of sulphates ; it is also increased by the action of certain poisons on the proteid metabolism—in acute poisoning by arsenic, chloroform, chloral hydrate, and in the second stage of acute phosphorus poisoning, the proteid tissues rapidly succumb and yield much sulphur. In diseases which are accompanied by rapid tissue metabolism, as in small-pox, typhus, pneumonia, and in rheumatic fever, an increase has been observed, but not invariably. In diabetes a large increase usually occurs without the $\text{N} : \text{S}$ quotient being disturbed ; this is due to the excessive amount of animal food that is eaten. Reale and Velardi¹ state that in diabetes the neutral sulphur is increased in excess of the oxidised sulphur, and that when the total sulphur is not increased the neutral sulphur is. In advanced Bright's disease, and especially in amyloid kidney, sometimes also in acute nephritis, the excretion of sulphuric acid has been found to be diminished.

(b) Under normal conditions about 0.1 gm. to 0.25 gm. of ether-sulphuric acid is excreted daily in the urine ; the relation of (A) preformed sulphates to (B) ether-sulphates is about 10 : 1. This ratio is subject to great fluctuations, chiefly determined by the activity of the putrefactive processes which take place in the intestinal canal. Vaughan Harley² found, after removing the large intestine in dogs, that whilst the excretion of the total sulphates corresponded with that of normal dogs the amount of ether-

¹ *Arch. f. Verdauungskrankh.*, 1896.

² *Proc. Royal Soc.*, 1898.

sulphates was reduced to one half, showing that the intestinal putrefaction was very much diminished. Intestinal putrefaction favours the formation of phenols, indol, and other aromatic products which conjugate with the oxidised sulphur; so that the relation borne by the ether-sulphates to the preformed sulphates affords a measure of the intensity of the intestinal putrefaction, as also of the occurrence of putrefaction elsewhere, and is of much greater diagnostic importance than a mere increase or decrease in the total excretion of sulphuric acid.

Apart from diseased conditions, mere diminution in the acidity of the gastric juice produces an increase in the proportion of ether-sulphates; hence the habitual use of sodium bicarbonate by dyspeptics, whilst alleviating gastric pain, increases the tendency to flatulence in the intestines by favouring putrefactive processes. On the other hand, the administration of hydrochloric acid materially lessens the excretion of ether-sulphates (Biernacki¹), as also does calomel, by diminishing the amount of intestinal decomposition. Biernacki states that milk-diet diminishes the formation of ether-sulphates, as it affords a medium that is unfavourable for intestinal putrefaction. The same pathological conditions that cause excessive formation of indoxyl (*q.v.*) also promote the formation of ether-sulphates; these conditions comprise various intestinal derangements which are causative of decomposition of the intestinal contents: tuberculosis, malignant disease, typhlitis, peritonitis, intestinal catarrh, absence of bile, stoppage of the bowels, and also, sometimes, obstinate constipation; in bacterial diseases as typhus, typhoid, and scarlet fevers, in small-pox, and occasionally in erysipelas, an increase occurs. In extreme instances the ether-sulphates may amount to 0.5 or 0.6 grm. per day; in poisoning by carbolic acid, the whole of the sulphuric acid may appear in the form of ether-sulphuric acid. In septic conditions apart from the intestines, as in empyema, or in other large collections of pus which is undergoing bacterial decomposition, the amount of ether-sulphates in the urine is increased.

(c) NEUTRAL SULPHUR.

Under this designation is comprised the unoxidised, or partially oxidised, sulphur which is furnished by a number of sulphur-containing bodies, such as sulphurous acid, sulphocyanides, the derivatives of taurin and cystin, melanogen (Stokvis²), ethyl sulphide, methyl

¹ *Centralbl. f. d. med. Wissensch.*, 1890.

² *Nederl. Tijdschr. v. Geneesk.*, 1899.

mercaptan, oxyprotinic acid, and the proteids of normal urine. It is usually accepted that the amount of neutral sulphur in the urine is determined by the degree of proteid decomposition which takes place in the organism; it has been estimated at from 16.3 per cent. (Salkowski¹) to 25.5 per cent. (Munk²), of the total sulphur of the urine. In hunger, it has been found as high as 70 per cent. (Tucek, Jerome³). Harnach and Kleine⁴ consider that the amount of neutral sulphur is so intimately dependent on the quantity and kind of food as to render investigations on the subject useless as aids to diagnosis. Benedict⁵ considers that the proportion of neutral sulphur excreted in urine is much less dependent on the amount of proteid metabolism than is the case with the sulphuric acid-sulphur, and that the amount of neutral sulphur oscillates between narrow limits, whether the proteids which furnish it are derived from food or from the tissues; the splitting up of the tissue proteids as such, causes no absolute increase in the unoxidised sulphur. He does not regard the neutral sulphur as intermediate and antecedent to sulphuric acid; although when a large amount of fat is also metabolised, a portion of the neutral sulphur may be converted into the fully oxidised acid. In children at the breast, Freund⁶ states that the neutral sulphur shows far less variations than either the oxidised or the total sulphur; the healthy children whose urine he examined excreted more neutral sulphur than those that were ill-nourished.

The discrepancies in some of these results are probably due to the different sources whence the neutral sulphur of the urine is derived; and also, to no inconsiderable extent, to the methods used in the investigations, some of which were conducted on animals and some on the human subject. As pointed out by Jerome, the kind of animal and the individual peculiarity, exercise no inconsiderable influence on the amount of neutral sulphur that is excreted.

Biernacki⁷ found, in icterus, that the neutral sulphur is increased and the sulphuric acid decreased; when the icterus is of long standing the neutral sulphur diminishes. Schmidt⁸ found a considerable increase in four cases of anæmia. The neutral sulphur has also been found to be increased in diabetes, in pneumonia, and in hunger.

One source of neutral sulphur—sulphurous acid—claims a few words. It is probably not always present in normal urine, and when

¹ *Zeitschr. f. physiol. Chem.*, 1885.

² *Archiv. f. Physiol.*, 1895.

³ *Pflüger's Arch.*, 1895.

⁴ *Zeitschr. f. Biol.*, 1899.

⁵ *Zeitschr. f. klin. Med.*, 1899.

⁶ *Zeitschr. f. physiol. Chem.*, 1899.

⁷ *Arch. f. klin. Med.*, 1893.

⁸ *Zeitschr. f. klin. Med.*, 1898.

present it is in exceedingly small amount. Presch¹ obtained 0.004 of sodium hyposulphite from a litre of urine. Strümpell² found sulphurous acid in the urine from a case of fever.

(d) SULPHURETTED HYDROGEN.

Sulphuretted hydrogen has exceptionally been found in freshly voided urine, as the result of certain abnormal conditions: From a special kind of fermentation set up in the bladder by micro-organisms, which have been variously described as diplococci (v. Jaksch³), as like typhoid bacilli (Karplus⁴) and other varieties; when tried experimentally, however, the micro-organisms found do not always develop sulphuretted hydrogen in sterile urine. It is supposed on the one hand that the gas is produced from the neutral sulphur that may be present in the urine; on the other hand, Goldmann⁵ found that the neutral sulphur is not diminished, whereas the total sulphuric acid is diminished. During the earlier stages of the formation of sulphuretted hydrogen the urine retains its acid reaction. In some instances, sulphuretted hydrogen is not developed in the urine, but is conveyed to it through a fistulous communication between the bowel and the bladder, or, alternatively, by diffusion of the gas in the same direction through the intact walls; it is also assumed that the gas may be absorbed from the intestine by the blood and excreted by the kidneys.

After acidulation with a mineral acid, any urine may be made to yield sulphuretted hydrogen by the application of warmth.

DETECTION AND ESTIMATION OF SULPHUR COMPOUNDS.

Detection of sulphuric and ether-sulphuric acids—To a little of the urine in a test-tube acetic acid is added to strong acid reaction in order to prevent the precipitation of phosphates; on dropping in a solution of barium chloride, a white precipitate indicates the presence of sulphuric acid. If, after filtering off the precipitate, the filtrate is further acidulated with hydrochloric acid and warmed, any ether-sulphuric acid that is present is liberated from its combinations and the free acid forms a second precipitate with the excess of barium chloride that remains in solution.

¹ Virchow's *Arch.*, 1890.

³ Jaksch, *Klin. Diagnostik*, 1896.

⁵ *Zeitschr. f. physiol. Chem.*, 1885.

² *Arch. d. Heilk.*, 1876.

⁴ Virchow's *Arch.*, 1893.

Estimation of total sulphuric acid.—Fifty cubic centimetres of urine are diluted with an equal volume of distilled water, and 10 c.c. of hydrochloric acid are added. The solution is raised nearly to the boiling-point and a solution of barium chloride is added in slight excess. The mixture is kept warm for an hour or two and is then allowed to stand in the cold for twenty-four hours, when it is passed through a small ash-free filter. The precipitate on the filter is well washed with distilled water, until the water gives no turbidity with sulphuric acid, nor silver nitrate, and then with alcohol; after which the filter is dried and burnt in a platinum crucible, the residue is ignited, and after cooling over sulphuric acid is weighed: 100 parts of barium sulphate = 41.99 of sulphuric acid.

In making this estimation, it is necessary to heat the urine nearly to 100° C. in order to liberate the conjugated acid, and also to render the barium sulphate crystalline; newly precipitated barium sulphate is in a state of such extremely fine division that it will pass through any but the densest filter-paper, which is not suitable for the present purpose; by boiling, or by keeping the urine for some time just below the boiling-point, the particles aggregate and are easily kept back by an ordinary filter. It is to be borne in mind, however, that barium sulphate is slightly soluble in dilute hydrochloric acid, and that this tendency would be increased by prolonged boiling.

Estimation of ether-sulphuric acid.—This is best made by Salkowski's¹ method.

To 100 c.c. of the urine is added an equal volume of a solution composed of two volumes of a saturated solution of barium hydrate and one volume of a saturated solution of barium chloride; after standing for a short time, the solution is filtered through a close-textured paper, and 100 c.c. of the filtrate, which corresponds to 50 c.c. of the urine, is strongly acidulated with hydrochloric acid, is heated nearly to 100° C., and the precipitate which is thrown down is dealt with as described for the estimation of the total sulphuric acid. The weight of the barium sulphate indicates the amount of (B) the ether-sulphates; if this is subtracted from the measure of the total sulphuric acid, the proportion of the (A) sulphuric acid is ascertained.

Neutral sulphur.—The easily separable sulphur may be estimated by Schulz's² process, which is founded on the following principles. When urine, or other fluid, which contains loosely combined sulphur, is boiled with soda and lead acetate, no lead sulphide

¹ Virchow's *Arch.*, 1880.

² *Zeitschr. f. physiol. Chem.*, 1898.

is formed, because, as the sulphur is separated, it is oxidised by the atmospheric oxygen. If, however, zinc in a state of fine division is added it acts as a reducing-agent, and then the unoxidised sulphur attacks the lead. The process is thus carried out: To 100 c.c. of urine in a flask, that is fitted with a reflux condenser, 50 c.c. of a 30 per cent. solution of sodium hydrate are added, along with a few drops of a concentrated solution of lead acetate, and about 1 grm. of fine zinc filings, free from sulphur. The mixture is boiled on a sand-bath for ten or eleven hours, when the easily separated sulphur will be found to have combined with the lead. The solution, acidulated with acetic acid, is filtered, and both precipitate and filter, after washing, are melted with three parts of soda and two of saltpetre; the fused product is dissolved in water and carbon dioxide is passed through it; it is then filtered, treated with hydrochloric acid—to drive off the nitric acid—and evaporated to dryness. The residue, dissolved in water, is precipitated with barium chloride, and the resulting barium sulphate is dealt with in the usual manner and weighed.

Sulphuretted hydrogen.—The odour of the gas is perceptible, and its presence may be further demonstrated by pouring some of the urine into a flask, the cork of which is nicked at its lower end in such a manner as to clip a piece of filter paper so that it hangs down the neck of the flask without touching its sides. The paper, which is moistened with a solution of lead acetate and then with a solution of caustic soda, becomes blackened from the formation of lead sulphide.

PHOSPHORIC ACID. H_3PO_4 .

Phosphoric acid is a tribasic acid, and consequently forms three classes of salts in which one, two, or the entire three atoms of hydrogen are respectively replaced by a metal. The dihydric salts turn blue litmus-paper red; the monohydric and the normal salts turn red litmus-paper blue; in other words, the former have an acid reaction and the latter two an alkaline reaction. When the acid-reacting and the alkaline-reacting salts are present in certain proportions the urine has an amphoteric reaction—it turns blue litmus-paper purple-red and red litmus-paper violet-blue. The alkali-salts of all three classes are freely soluble in water; the dihydric earthy-salts are sufficiently soluble to remain as such in solution in urine; the monohydric earthy-salts are less soluble, and the normal earthy-salts are still less soluble in aqueous liquids. When in solution, the monohydric earthy phosphates are decomposed by heat into the dihydric and the normal salts, the former remain in solution

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and the latter are precipitated. If, after boiling, the solution is allowed to stand, it tends to return to its original condition, the precipitated normal salt being gradually redissolved; this occurs quickly if, in place of boiling, the solution is merely heated sufficiently to produce the decomposition.

From 2 to 3.5 grms. of phosphoric acid, in combination with lime, magnesia, and the alkalies, are excreted daily in the urine of adults. In relation to the nitrogen excretion the proportion of $N:P_2O_5$ is about 5 or 6:1. In normal urine Ott¹ found an average ratio of six parts of dihydric phosphates to four of the monohydric. A very small amount of organically combined phosphorus is also present in the urine; the daily average is stated by Ceconi² to be from 11 to 28 mgrms. About one-third of the ingested phosphorus is excreted by the bowels. When much lime is taken, either apart or contained in food, the amount of phosphoric acid in the urine is diminished, and the insoluble calcium salts that are formed are excreted in the fæces. The amount of phosphoric acid is largely determined by the nature and the quantity of the food. P_2O_5 is not furnished by ordinary proteids, but by tissues that are rich in nuclein. By administering the thymus of the calf, free from albumen and peptone, to dogs, Gumlich³ found that more than one-half of the ingested nucleinic acid phosphorus reappeared in the urine. The most important constituent of the cell-nuclei contains organically combined phosphorus which, when taken as food, is retained by the tissues, whilst they neglect similarly administered metallic phosphates; yet, when nuclein-containing food is ingested, the urinary phosphoric acid is increased to a greater extent than the amount of phosphorus contained in the nuclein will account for. The investigations of Milroy and Malcolm⁴ tend to show that the digestive products of nuclein-containing substances cause hyper-leucocytosis, which is accompanied, or followed, by temporary destruction of white blood-corpuscles. Their observations show that the excretion of phosphoric acid in the urine is both absolutely and relatively increased by the ingestion of small doses of nucleinic acid. Thus, on a fixed diet for two periods of eight days, the $N:P_2O_5$ quotient, during one of the periods without nucleinic acid, was 5.12:1; whilst during the second period in which nucleinic acid was given the quotient was 3.7:1. On the other hand, when metaphosphoric acid was given under like conditions, the increase in the urinary phosphates did not equal the amount of HPO_3 administered, the balance being excreted in the fæces. In infants

¹ *Zeitschr. f. physiol. Chem.*, 1886.

² *Congres. f. inn. Med.*, 1896.

³ *Ibid.* 1894.

⁴ *Journ. of Physiol.*, 1898.

at the breast, Keller¹ found the variations in the urinary phosphates to be much greater than the phosphoric acid value of the different kinds of milk on which they were fed. He gives the ratio of N to P_2O_5 as 3.3 : 1 for human milk, and 2.3 : 1 for cows' milk; yet the urine of the child at the breast gave a ratio of 7 : 1, whilst when fed by hand it was 1.7 : 1. The explanation is afforded by the respective proportions of organically combined phosphorus in the two milks: in human milk the combined phosphorus amounts to 41.5 per cent. of the total phosphorus it contains; in cows' milk it is only 6 per cent. (Siegfried²). From these experiments it would appear that, in the infant, whilst the combined phosphorus is retained, the leucocytosis is not accompanied by leucolysis.

It is probable that the organically combined phosphorus in the urine is solely derived from the metabolism of the nuclein-containing tissues, and that its amount is not influenced by the ingestion of food rich in nuclein. Mandel and Oertel³ examined the urine of three young men who, for periods of three and four days, lived exclusively on phosphorus-free and on phosphorus-rich diet; no significant variation was found. These experiments agree with the results previously obtained by Ceconi, Keller,⁴ and Loewi.

It is usually stated that the excretion of phosphoric acid is increased by prolonged muscular activity; the result probably depends partly on the duration and the severity of the exercise, and partly on the state of nutrition of the individual upon whom the observations are made. Dunlop,⁵ along with Paton, Stockman, and Maccadam, in the course of some investigations on the effects on metabolism produced by excessive work, found that when the individual was in good condition no increase in uric acid, extractive nitrogen, nor in phosphorus occurred; but when he was in poor training these substances appeared in the urine in increased amount. The explanation given is that under the condition first named the increase in metabolism fell chiefly on the muscles, which are poor in nuclein; whilst in the feebler subject, other tissues which contain nucleo-proteids are laid under contribution. Garratt⁶ found a small increase in the urinary phosphoric acid after, but not during, a period of active exercise short of excessive fatigue.

Under conditions in which rapid metabolism of the nuclein-containing tissues occurs, as in acute phosphorus poisoning, the amount of phosphoric acid in the urine is increased. In meningitis, especially in children, it is greatly increased; also in the early stage of

¹ *Zeitschr. f. klin. Med.*, 1898.

³ *N.Y. Bull. Med. Sc.*, 1901.

⁵ *Journ. of Physiol.*, 1898.

² *Zeitschr. f. physiol. Chem.*, 1896.

⁴ *Arch. f. Kinderheilk.*, 1900.

⁶ *Ibid.*

osteomalacia Neumann¹ found much P_2O_3 in the urine; whilst in the later stage, during the progress of recovery, it was retained to form bone. It has also been found to be retained in severe arthritis deformans. In profound anæmia, v. Noorden² found great excess; but in spleno-medullary leucocythæmia Milroy and Malcolm³ found a decrease, both absolutely and also relatively in proportion to the total nitrogen. A like result was obtained by Hale White and Hopkins⁴ in splenic leucocythæmia. During prolonged inanition, and in the course of some fevers, the excretion of phosphoric acid is diminished. Ceconi⁵ found this to be the case in severe fevers associated with dyspnœa, and Moraczewski⁶ also found diminution in the earlier stages of fever, with subsequent increase. In pneumonia Hutchison⁷ found an increase during the febrile stage with a diminution after the crisis; occasionally, a further increase occurred about the third or fourth day of convalescence. In diabetes the output of phosphoric acid is only excessive in severe cases. In the so-called phosphatic diabetes enormous quantities are excreted, as much as from 15 to 25 grms. in the twenty-four hours.

Many fresh urines that have an alkaline, or a faintly acid reaction, deposit earthy phosphates spontaneously, or when they are boiled. This condition is often met with in neurasthenic patients, and, by erroneous interpretation, is frequently spoken of as phosphaturia, from the supposition that it indicates an excess of phosphoric acid in the urine, arising from undue degeneration of the cerebral and other nerve tissues; but it so happens that in such cases the phosphoric acid may be present in less than the normal amount. The most common causes of this condition are, diminished excretion of phosphoric acid or increased excretion of the earthy bases; excess of alkali will also produce it. In two cases of neurasthenia Panek⁸ systematically examined the urines which over a considerable period deposited earthy phosphates. He found that the excretion of phosphoric acid was reduced to 1.8–2.1 grms. daily, whilst that of the calcium was increased to 0.51–0.56; the magnesium excretion was not materially altered, and organically combined phosphorus was only present in traces. The nitrogen excretion in the urine was diminished throughout the course of these cases.

The presence of phosphoric acid in acid urine is demonstrated by the precipitate of earthy phosphates which falls on the addition of a little ammonia. When the urine is alkaline it should be acidified

¹ *Ungar. Arch. f. Med.*, 1894.

³ *Loc. cit.*

⁵ *Morgagni*, 1898.

⁷ *Journ. of Path. and Bacteriol.*, 1898.

² *Pathol. d. Stoffwechsels*, 1893.

⁴ *Journ. of Physiol.*, 1900.

⁶ *Virchow's Arch.*, 1899.

⁸ *Przegląd lekarski*, 1900.

with acetic acid and then treated with a few drops of a solution of ferric chloride, which produces a yellowish white precipitate of ferric phosphate.

Estimation.—Neubauer's¹ method, slightly modified, is the one usually adopted. The following solutions are required:

(a) A solution of sodium phosphate that contains 0.1 gm. of P_2O_5 in 50 c.c. This is prepared by dissolving 10.0845 grms. of sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$) in distilled water and making up to a litre. The salt must be pure, and must be quite free from chlorine.

(b) 100 grms. of sodium acetate are dissolved in distilled water, and 100 c.c. of 30 per cent. acetic acid are added, and then the solution is made up to a litre.

(c) 20.3 grms. of pure uranium oxide are dissolved in pure acetic acid and then made up with water to the litre: 1 c.c. corresponds to 0.005 gm. of P_2O_5 .

(d) An infusion or tincture of cochineal.

The strength of the uranium solution is first tested by titration. Into a porcelain dish 50 c.c. of the phosphate solution, 5 c.c. of the sodium acetate solution, and a few drops of the tincture of cochineal are put, and are heated to boiling; into the boiling mixture, with constant stirring, the solution of uranium is run in from a burette until a permanent green coloration is produced, which is the end-reaction. The strength of the uranium solution must be adjusted until 20 c.c. exactly corresponds to 50 c.c. of the phosphate solution.

The estimation of the phosphoric acid in the urine is then made in precisely the same way: 50 c.c. of urine, 5 c.c. of (b) with a few drops of (d) are boiled in a dish and the solution (c) is run in, with constant stirring, until the green end-reaction is obtained. Each c.c. of (c) represents 0.005 gm. P_2O_5 .

Hydrofluoric and silicic acids may be present in urine in very minute traces, obviously introduced in food. Their presence is of no practical moment.

CARBON DIOXIDE. CO_2 .

Carbon dioxide occurs in urine in the free and in the combined conditions. The free carbon dioxide is present in simple solution, and is produced by the action of the dihydric phosphates on the urinary carbonates. The amount dissolved in urine is very variable, and to a great extent is dependent on the kind of food that is eaten; large quantities of vegetable foods determine an increase both in the

¹ *Arch. f. wissenschaft. Heilk.*, 1859.

combined and in the free carbon dioxide. From 50 c.c. to 400 c.c. of the free acid have been obtained from the twenty-four hours' urine; with an exclusively vegetable diet as much as 600 c.c. may be present. The combined carbon dioxide may be present in the form of carbonates, in such large amount as to cause the urine to effervesce on the addition of an acid.

Detection.—A stream of air, which has been previously passed through a solution of potash in order to free it from carbon dioxide, is drawn through the urine and then through a clear solution of barium hydrate for several hours; any turbidity that results is due to the formation of barium carbonate. If the barium solution is of known strength, the amount of carbonate formed may be ascertained by subsequent titration. It is scarcely possible to remove all free carbon dioxide from the urine; because, as it is removed, more is developed by the action of the dihydric phosphates on the carbonates.

After all, or as much as possible of the free carbon dioxide has been exhausted, the addition of an acid to the urine liberates the combined carbon dioxide, which may also be determined in the above described way.

Nitric acid (HNO_3), combined with some of the inorganic bases, is present in very small amount in normal urine. After urine has stood for some time, the nitric acid will probably be reduced to nitrous, on account of a kind of fermentation which is set up by micro-organisms. On distilling urine with one-fifth its volume of sulphuric acid, any nitric acid present comes over in the distillate as nitrous acid. If to the distillate, some solution of sulphanilic acid and naphthylamine, along with a trace of sodium nitrite be added, and the mixture is then warmed, it acquires a red colour.

Hydrogen peroxide (H_2O_2) was found in urine by Schönbein,¹ but its presence is not capable of satisfactory demonstration by ordinary clinical methods. It is recommended that sufficient of a solution of indigo should be added to some fresh urine as to produce a green coloration; the urine is then divided into two portions, to one of which a dilute solution of ferrous sulphate is added; if hydrogen peroxide be present the green colour becomes lighter, or disappears. The other portion of urine retains its green colour and is used for the purpose of comparison.

Oxygen has been found, as a trace, in urine. Berthelot,² however, attributes the statement to erroneous methods of research; his experiments show that, after being voided, urine may absorb from 30 to 40 c.c. of oxygen per litre.

¹ *Journ. f. prakt. Chem.*, 1864.

² *Compt. Rendus*, 1900.

BASES.

POTASSIUM AND SODIUM.

IN the normal condition, from 2 to 4 grms. of potash and from 4 to 7 grms. of soda are daily excreted in the urine by adults; according to Munk,¹ the proportion of potash to soda is as 144 to 317. The amount is chiefly determined by the quantity and the character of the food. In inanition both are reduced, and in the absence of common salt the normal proportion of potash to soda is inverted; the potash of the urine is largely dependent on the tissue-metabolism, and consequently is less influenced than soda is by food. Dunlop found,² when excessive exercise is associated with sweating, that the output of soda is diminished, that of potash remaining unaltered; under like conditions Munk found the potash to be increased. In fevers an excess of potash with marked diminution of soda, sometimes to exclusion, takes place; in the convalescent stage the soda rapidly increases and the potash sinks below normal (Salkowski³). In cases of nephritis about to terminate fatally, Herringham⁴ found complete, or almost complete, absence of sodium in the urine; in the same disease Charrier⁵ found gradual and progressive retention of potassium.

Estimation.—Lehmann's⁶ process, by which good results are obtained, is as follows: To 100 c.c. of urine 4 grms. of ammonium sulphate are added, and the mixture is evaporated to dryness; the residue is fused in a platinum basin, a little sulphuric acid being added, if needed, so as to thoroughly oxidise the organic matter and leave the residue quite white. The residue is dissolved in hot water which contains a little hydrochloric acid, and, whilst boiling, a solution of barium chloride is added to complete precipitation; then, whilst hot, the liquid is supersaturated with ammonia and ammonium carbonate. After standing and filtering the filter is washed, and the filtrate and the wash-water are evaporated to a small volume; this solution should give no precipitate with ammonia and ammonium carbonate. It is then, at a temperature below 100° C., evaporated to dryness in a tared platinum crucible, and, in order to drive off the ammonia the residue is carefully heated in the covered crucible for a considerable time, nearly, but not quite, to red heat; great care must be taken not to allow the platinum to become red hot, else

¹ Virchow's *Arch.*, 1893.³ Virchow's *Arch.*, 1871.⁵ *Compt. rend. Soc. Biolog.*, 1897.² *Journ. of Physiol.*, 1898.⁴ *Brit. Med. Journ.*, 1903.⁶ *Zeitschr. f. physiol. Chem.*, 1884.

some of the chlorides will be volatilised. After cooling in a desiccator the combined chlorides are weighed.

In order to do away with the risk of loss by driving off the ammonia in the dry state, Herringham¹ modifies the later stage of Lehmann's process by neutralising with sodium hydrate instead of ammonia, and precipitating the barium with sodium carbonate; the filtrate is boiled to drive off the ammonia, and after neutralising with hydrochloric acid it is again boiled to drive off the carbon dioxide. The solution is evaporated to dryness and the combined chlorides are weighed, the amount of soda that was added being deducted. When the potash alone is to be estimated, Herringham, after neutralising with hydrochloric acid and driving off the carbon dioxide, concentrates the solution to 100 c.c., adds 30-50 c.c. of a 10 per cent. solution of platinic chloride, and then evaporates to dryness. After cooling, the sodium double salt is dissolved out with alcohol and the insoluble potassium double salt is dried and weighed.

Pribram and Gregor² thus modify the first stage of Lehmann's process: To 50 c.c. of urine, 10-20 c.c. of a 10 per cent. solution of barium permanganate, and 10 c.c. of sulphuric acid (1:10) are added, and the mixed liquids are boiled. If the red colour quickly disappears, a little more of the permanganate solution is added, so that the colour only gradually disappears after 10 to 15 minutes' boiling; any excess of permanganate is removed by a little oxalic acid. The solution thus obtained is precipitated with barium chloride, and is subsequently dealt with as in Lehmann's process.

AMMONIA.

A small and varying proportion of the urinary nitrogen occurs in the form of ammonia salts; ammonia N : total N = about 1 : 24. In health and with ordinary diet the ammonia present in urine ranges from 0.5 to 1.5 grms. in the twenty-four hours; this represents from 3.5 to 6 per cent. of the total nitrogen; with a diet rich in proteids, the quantity of food at the same time being insufficient, the ammonia N to total N quotient, reached 1 : 13 (de Groot³).

In the normal state the amount of ammonia excreted in urine is determined by the supply of bases yielded by the food which is eaten; if the food is poor in bases and consequently the acids are in excess, as when large quantities of animal food are eaten, more of the excreted nitrogen than otherwise would be the case, comes away in

¹ *Journ. of Physiol.*, 1898.

² *Zeitschr. f. physiol. Chem.*, 1899.

³ *Nederl. Tijdschr. v. Geneesk.*, 1899.

the form of ammonia with which the acids combine, and the ammonia salts thus formed appear in the urine. On the other hand, excess of vegetable food, which is rich in bases, diminishes the proportion of ammonia; the same result follows the administration of the fixed alkalies. It will thus be seen that ammonia is increased at the expense of the urea, so that the balance of nitrogen excretion is maintained. Increased muscular work causes an excessive excretion of ammonia, amounting to 1.18 grms. compared with 0.87 grm. during rest (v. Noorden).

Under pathological conditions the percentage of ammonia is increased in malaria, in fevers (6–7 per cent.), in pneumonia, pleurisy, cholera, rheumatoid arthritis (Rumpf¹), in diseases of the liver, such as cirrhosis, malignant disease, acute atrophy, fatty and phosphorus liver, and in malignant disease, especially of the abdomen. In extrinsic acid poisoning there is excess, and also in intrinsic acid poisoning, or acidosis, due to faulty metabolism as in diabetes (2 to 4 grms. in the twenty-four hours) when accompanied by the formation of diacetic and β -oxybutyric acids. v. Noorden believes that, in Bright's disease, but not in health, vapour baths increase the elimination of urea and diminish that of ammonia. Köhler² disputes this, and states that the alleged difference between healthy people and those suffering from Bright's disease is not proved. In pernicious anæmia the percentage of ammonia in the urine may be considerably above, or it may be rather below, the average. Blumenthal³ thus classifies the conditions under which ammonia is formed in the organism: Lessened synthesis of urea as occurs in diseases of the liver; acidosis, as in diabetes and extrinsic poisoning; bacterial decomposition of tissue-proteids, as in fevers.

Estimation.—On account of the readiness with which urea undergoes hydrolysis, it is not easy accurately to estimate the amount of ammonia present in urine. Schlösing's method is commonly adopted. A ground-glass plate is fitted with a bell-glass, ground at its mouth and smeared with tallow. In the centre of the plate is placed a small, flat-bottomed, glass basin or shallow beaker, which contains 10 c.c. of decinormal sulphuric acid; over the beaker is a triangle which supports a smaller shallow beaker containing 20 c.c. of the urine. An equal volume of milk of lime is added, and the bell glass is placed over the beakers so as to form an air-tight chamber. The apparatus is left for forty-eight hours, when the acid is titrated, and the amount of ammonia it has absorbed is thus ascertained. The milk of lime liberates the ammonia which is present in simple

¹ Virchow's *Arch.*, 1896.

² *Deutsches Arch. f. klin. Med.*, 1900.

³ *Pathol. d. Harnes*, 1903.

combination, but does not attack the urea and other nitrogenous constituents. The results obtained, however, are rather too low.

The procedure may be shortened and rendered more accurate by distilling the urine *in vacuo*; for this, the apparatus of Nencki and Zaleski¹ may advantageously be used. Krüger and Reich² add a little alcohol to the urine, before distillation, in order to lower the point of ebullition and diminish frothing.

CALCIUM AND MAGNESIUM.

The alkaline earths are introduced into the system in food, and are partly excreted in the urine and partly in the fæces. On a mixed diet, the daily excretion of urinary calcium is from about 0.25 to 0.35 grm. that of magnesium is rather less, from 0.15 to 0.25 grm. Neumann³ gives the normal proportion of lime to magnesia, excreted in the urine, as about 3:1. v. Noorden states that, in healthy people, from 4 to 29 per cent. of the ingested lime appears in the urine; the remainder is excreted in the fæces. Proportionately less magnesia than lime is excreted by the bowels. In inanition, Munk⁴ found that there is an increase in the excretion of lime, more than the metabolism of the systemic proteids, as shown by the nitrogen excretion, will account for; the inference being that, in starvation, the bones part with some of their lime. Observations on the extent to which the alkaline earths are excreted in the urine, in pathological conditions which affect the bones, are somewhat discordant. In rickets, for example, Baginsky⁵ and Rüdel⁶ found no marked influence; on the other hand Babeau⁷ found that, during the developmental period of rickets, both the calcium and the magnesium of the urine were increased; later on, when the process was at a standstill, the excretion of both approached the normal. He regards increased excretion of lime in the urine as an expression of the absorption of bone-substance; whilst an increase of lime in the fæces is due to deranged absorption of the lime present in the food. In a case of hypertrophic osteo-arthritis, Guérin and Etienne⁸ found an increase in the daily urinary excretion of calcium; at a later period it fell considerably—to 0.094 grm.; the magnesium excretion did not materially differ from the normal. In a case of arthritis deformans 1.28 grms. of lime and 0.06 magnesia were retained daily; in chronic rheumatism the excretion is normal. In diabetes, Gerhard and Schlesinger⁹ state that the excretion of calcium

¹ *Arch. f. exp. Pathol.* 1895.

³ *Ung. Arch. f. Med.*, 1894.

⁵ *Ibid.* 1882.

⁷ *Compt. Rendus*, 1898.

⁹ *Arch. f. exp. Pathol.*, 1899.

² *Zeitsch. f. physiol. Chem.*, 1903.

⁴ *Virchow's Arch.*, 1893.

⁶ *Arch. f. exp. Pathol.*, 1893.

⁸ *Arch. de méd. expér.*, 1896.

runs a parallel course with that of ammonia, as it does in the healthy condition; during acidosis, therefore, the excretion of calcium is very high. In severe diabetes when the alkalies are used up by the excess of acids, the bones part with some of their lime and magnesia, and the normal relation of urinary and faecal excretion is altered—most of the lime appears in the urine, and it may be increased to tenfold over the normal. In mild cases of diabetes no alteration takes place. In calcareous degeneration of the arteries, Rumpf¹ found that the excretion of lime is diminished.

The presence of the earthy salts in the urine is indicated by the precipitate which falls on the addition of a little ammonia; it consists chiefly of calcium and ammonio-magnesium phosphates. The precipitate is soluble in acetic acid.

Estimation. Calcium.—To 200 c.c. of urine a little ammonia is added; the precipitate which forms is dissolved by the addition of a bare sufficiency of hydrochloric acid, and an excess of ammonium oxalate is added, along with some sodium acetate. The solution, in a covered beaker, is heated on the water-bath for ten or twelve hours. The precipitate of calcium oxalate which forms is filtered off on an ash-free filter, washed free from chlorine and dried. The filter, with the precipitate retained, is burnt in a platinum crucible and the residue is strongly ignited for about ten minutes until it is quite white. After cooling, the calcium oxide is weighed: 1 part = 1.845 parts of $3\text{CaO}, \text{P}_2\text{O}_5$.

Magnesium.—Another 200 c.c. of the same urine are treated as above, and after the precipitate of calcium oxalate has been filtered off the filtrate is treated with ammonia, by which the magnesium is precipitated as magnesium and ammonium phosphate. This is collected on an ash-free filter and is washed with weak ammonia water and dried. The filter is then burnt in a platinum crucible (preferably after as much as possible of the precipitate has been transferred to the crucible) and the precipitate is ignited at a high temperature until it is colourless. After cooling, the residue, which consists of magnesium pyrophosphate, is weighed: 1 part = 0.36208 part of MgO .

IRON.

A very small amount of organically combined iron is always present in normal urine; it has been variously estimated from as low as 0.5 mgrm. (Hall²), up to 8 mgrms. (Jolles and Winkler³), in

¹ *Verhandl. d. Congres. f. inn. Med.*, 1897.

² *Arch. f. Anat. u. Physiol.*, 1894.

³ *Arch. f. experim. Pathol.*, 1900.

the twenty-four hours' urine. On mixed diet Bartoletti¹ found 2.89 mgrms., and on animal diet 5.19 mgrms.; he also found that there is no constant relation between the amount of iron excreted in the urine and that contained in the blood. When iron is administered medicinally, barely a trace of it appears in the urine; it is excreted by the bowels. When injected subcutaneously the amount excreted in the urine is increased, but only on the first day after the injection (Bartoletti). The amount is increased in diseased conditions which are attended by hæmolysis; in pernicious anæmia (Hunter,² Hopkins³) and some other forms of severe anæmia; in diabetes with excretion of oxybutyric acid it has been found to be from six to seventeen times greater than the normal (Jolles and Winkler), also in malaria, contracting kidney, and after an attack of gout; on the other hand, it has been found in the normal amount in chlorosis, catarrhal jaundice, and alimentary glycosuria. Hoffmann⁴ found, in normal urine, an average daily amount of 1.09 mgrms., in phthisis 0.47 mgrm., in leucocythæmia 1.37 mgrms., in diabetes 3.7, and, in one case, in 5600 cc. of urine 22.02 mgrms.

Organically combined iron does not respond to the tests used for the detection of the metal; therefore, the organic substance must be destroyed and the iron left free before it can be identified. To detect the presence of iron in urine, 300–400 c.c. of the urine should be evaporated to dryness and the organic residue incinerated. The ash dissolved in a little hydrochloric acid and water, and boiled, after the addition of a drop or two of nitric acid, yields a red colour with a little solution of potassium sulphocyanide.

Estimation.—The urine, 300 or 400 c.c., is evaporated to dryness and the residue is carbonised over the Bunsen flame; after cooling, the carbonised product is warmed with hydrochloric acid on the water-bath; distilled water is then added and the solution is filtered through iron-free paper. The residue on the filter is well washed and is then transferred to a platinum basin, a little sulphuric acid is poured over it, and heat is applied to complete incineration. When cold the filtrate and the wash-water are poured over the incinerated product, a little sulphuric acid is added, the liquid portion is gently evaporated to dryness, and the residue is once more incinerated so as to ensure the destruction of all organic matter. The ash is then dissolved in dilute sulphuric acid with the aid of heat.

Any iron that is present in the solution thus obtained is in the ferric state and must be reduced to the ferrous state before

¹ *Riforma Med.*, 1902.

² *The Practitioner*, 1889.

³ *Guy's Hosp. Reps.*, 1894.

⁴ *Zeitschr. f. analyt. Chemie*, 1901.

being titrated. This may be accomplished by warming the solution with sulphurous acid in a flask from which air is excluded, the sulphurous acid being subsequently got rid of by passing pure well-washed, carbon dioxide for several hours through the solution, heated to nearly 100°C .

A more simple way of effecting reduction is described by Gintle¹: The ferric solution, acidified with sulphuric acid, is introduced into a flask furnished with a Bunsen valve, and into it is plunged a spiral of palladium that has been charged with hydrogen by heating it at 100°C . in a current of that gas, or if preferred by electrolytic means. After the solution has been heated with the palladium for about an hour and a half, on the water-bath, it is cooled and the spiral is withdrawn; the solution is then ready for titration.

Titration is effected by means of a solution of potassium permanganate, the titre of which, previously determined by titration with a solution of iron of known strength, should be low: 2 c.c. of permanganate solution may represent 1 mgrm. of iron. Into the flask containing the solution derived from the urine the permanganate solution is run from a burette, with constant agitation of the flask, until a permanent, faint rose colour is obtained; this is the end-reaction, indicating that all the iron has been oxidised. As the operation is one of extreme delicacy, the excess of permanganate required to clearly develop the end-reaction should be estimated; this is accomplished by adding to a volume of water equal to the volume of the iron-containing solution a sufficiency of the permanganate solution to produce a like depth of colour; the amount of permanganate thus used is deducted from that which was previously used.

Naumann² destroys the organic matter of the urine by heating it with one-tenth its volume of nitric acid, and subsequently with a mixture of sulphuric and nitric acids. The acid solution thus obtained is diluted with water, and a solution of zinc sulphate and sodium phosphate is added; the solution is then slightly alkalisied with ammonia and is boiled. A precipitate of zinc-ammonium phosphate is thrown down, which carries with it any iron that is present. The precipitate is dissolved in hydrochloric acid and titrated with potassium iodide; the amount of iodide set free is estimated by starch and a $\frac{\text{N}}{250}$ solution of sodium thiosulphate.

¹ *Zeitschr. f. angewandte Chem.*, 1902.

² *Arch. f. Physiol.*, 1902.

Zickgraf¹ separates the iron by precipitating it along with albumin—100 c.c. of urine are treated with 70 c.c. of a dilute solution of albumin; a little acetic acid is added, and the albumin is precipitated by boiling. The coagulated albumin, which has carried down the iron, is separated, dried, and incinerated; the iron is then dissolved and titrated in the usual way.

Zeitschr. f. analyt. Chem., 1902.

ORGANIC CONSTITUENTS.

NEUTRAL FAT.

CHYLE.

WHEN chyle is present the urine has the appearance of milk, or it may have a reddish tinge due to the admixture of a little blood. The milk-like appearance is caused by an emulsion of very finely divided fat; frequently so fine are the particles as not to be visible under a high microscopic power, but occasionally, along with granular matter, visible fat globules are present. On standing, the urine "creams" from the formation of a layer of fat globules on its surface. If the urine is shaken with ether the fat is dissolved and the milkiness disappears, giving place to a clear urine-like liquid, or some amount of turbidity may persist. After being voided, and sometimes before, chylous urine may coagulate and form a trembling jelly, either white like blanc-mange or it may be red-tinted; this, after a time, breaks up and is re-dissolved. The urine is acid, and has a specific gravity of about 1015 to 1020. It is coagulated by nitric acid and usually, but not always, by heat. It contains from 0.6 to 0.9 per cent. of proteids, 0.8 to 1.8 per cent. of fat, about 1.5 to 2 per cent. of urea, but no sugar. The urine that is voided early in the morning is often clear, the milky appearance only occurring after food has been taken. This is not always the case, as in some instances the night urine has been milky, the day urine being clear. Slosse¹ reports a case in which the day urine becomes chylous if the patient reclined on his back, but not when he lay prone.

The etiology of chyluria may be parasitic or non-parasitic. Parasitic chyluria is usually associated with the occurrence in human beings of the *Filaria sanguinis hominis*, though the precise way in which the parasite causes chyluria is not altogether understood. It appears probable, as held by Manson,² that the parasite invades the thoracic duct, and there sets up inflammatory processes which lead to stenosis of the duct. The chyle is thus prevented from reaching

¹ *Bull. Soc. Med.*, Bruxelles, 1901.

² *The Lancet*, 1892.

the subclavian vein, and is driven to find its way through the lymphatics of the abdomen and pelvis, which consequently (especially those that are not well supported by neighbouring tissues) become varicose; this is likely to be the case in the lymphatics of the bladder, and it is supposed that rupture of a varicose lymph channel takes place, through which the chyle escapes into the urinary tract.

The causes of non-parasitic chyluria are even less understood; no satisfactory anatomical explanation has yet been given. It has occurred in cases in which the only *post-mortem* appearances were: cancer of the posterior wall of the pyloric end of the stomach, abdominal tumours, and peritoneal adhesions. Meinert¹ relates a case of nocturnal chyluria which occurred in a pregnant woman, and which abruptly ceased after delivery.

Lipuria.—Under exceptional circumstances, apart from chyluria, fat has been found in the urine in sufficiently large globules as to be visible to the unaided eye, a condition to which the name *Lipuria* is given. This has occurred in diseases which involve extensive fatty changes in the kidneys; in some cases of ordinary chronic white kidney, in pyonephrosis (Ebstein²), and in acute phosphorus poisoning. Duckworth³ saw a man who had multiple sarcomatous growths, and whose urine on several occasions contained a large number of free fat globules. After death it was found that the fat came from small masses of the new growth, which were breaking down, in both kidneys. Southam⁴ reports the case of a man in whom fat-embolism took place after a compound fracture of the tibia. Four days after the accident minute fat globules were noticed floating on the urine, due to the passage of some of the fat from the medulla of the fractured bone into the open veins near to the seat of the fracture. It appears to be possible for free fat to pass from the digestive tract to the blood and thence through the kidneys; this occurred to a child a year and a half old after it had taken three teaspoonfuls each of castor and olive oils (Schlossmann⁵). Roberts⁶ also quotes two cases in which patients who were taking cod-liver oil subsequently passed a yellow oil with the urine. On one occasion, in a case of saccharine diabetes, the author found that the urine had a turbid appearance, which under the microscope was seen to be due to finely divided fat; on shaking with ether the urine at once became clear, and the ether yielded a small amount of fatty matter. In the course of some alimentary investigations this patient had been taking a large quantity of butter. In these cases it is probable that the oily

¹ *Centralbl. f. Gynæcol.*, 1902.

² *St. Bart. Hosp. Reps.*, 1885.

³ *Arch. f. Kinderheilk.*, 1894.

⁴ *Arch. f. klin. Med.*, 1879.

⁵ *The Lancet*, 1902.

⁶ *Urinary and Renal Diseases*, 1872.

substance passes through the kidney epithelium in a very finely divided state, and that the particles, being free, subsequently coalesce in the lower urinary passages and thus form globules that are visible to the unaided eye.

Two possible sources of contamination of urine with fatty matter must not be overlooked—the oil used to lubricate catheters, and fatty substances, including milk, that are occasionally purposely added to the urine by boys and hysterical women.

Detection.—Under the microscope chylous urine shows no fat globules, or only a few, whereas urine containing milk is crowded with them. When chylous urine is shaken with ether, the fat is extracted and the urine is left clear, or nearly so; when urine, to which milk has been added, is dealt with in the same way it remains unaltered. After removal of the fat, chylous urine should be tested in the usual way for albumin.

In lipuria the fat should be extracted with ether, and, after separation, the ether should be evaporated and the nature of the fatty matter ascertained by determining its melting-point.

CHOLESTERIN. $C_{27}H_{45}OH$.

Cholesterin is a fatty substance that has the characteristics of a monohydric alcohol. It is insoluble in water, is freely soluble in ether, chloroform, and boiling alcohol, and from alcoholic solution it crystallises in the form of large, thin, rhombic tables which have a mother-of-pearl-like lustre. Out of a solution in ether, or chloroform, it crystallises in the form of silky needles.

Cholesterin is very rarely found in urine; when it occurs, it is usually derived from a collection of pus that has been retained in a cavity for some time, which ultimately discharges into the urinary passages. It is also to be found in chyluria. Roberts¹ relates the case of a young man who suffered from symptoms of renal calculus, who passed blood-stained urine with crystals of cholesterin and free oily globules for many months. Murchison² saw a man who for fourteen years had passed large quantities of pus in the urine in which cholesterin crystals were found. At the necropsy both the kidneys were found to have been converted into suppurating cysts, on account of blocking of the ureters with calculi.

Separation.—The urine is extracted with alcohol-free ether which, on evaporation, leaves the cholesterin in an impure condition; this may be purified by dissolving it in strong alcoholic potash, with the aid of heat, evaporating to dryness, extracting the residue

¹ *Urinary and Renal Diseases*, 1887.

² *Trans. Path. Soc. Lond.*, 1868.

with ether, and, after evaporation of the ether, taking up the deposit in a small quantity of boiling alcohol, from which it crystallises in the characteristic rhombic tables.

If a little cholesterin is dissolved in a small quantity of chloroform and shaken with an equal volume of strong sulphuric acid, the chloroform becomes bright red in colour and the underlying layer of sulphuric acid acquires a greenish fluorescence. If the chloroform is then evaporated the residue first turns blue and subsequently green.

ORGANIC ACIDS.

VOLATILE FATTY ACIDS. $C_nH_{2n}O_2$.

VOLATILE fatty acids are present in very small amount in normal urine; acetic acid, and, in still less amount, formic and butyric acids may occur. In some febrile conditions, in diseases of the liver, and in diabetes, they have been found in larger amount, and occasionally propionic acid has also been found. For their detection a considerable amount of urine is required, which, after acidulation with 10 per cent. of syrupy phosphoric acid, is distilled as long as the distillate comes over with an acid reaction. The distillate is neutralised with sodium carbonate and evaporated to dryness. The residue is well extracted with hot, absolute alcohol, which dissolves the sodium salts of the fatty acids; after filtration the alcohol is driven off, and the residue, dissolved in water, is acidulated with a little sulphuric or phosphoric acid, is re-distilled, and portions of the distillate are tested for the several acids. The presence of formic acid is shown by adding a little solution of silver nitrate, which produces a white precipitate that quickly becomes black, especially by warming. With acetic acid, silver nitrate produces a white precipitate, which is not reduced by heat and consequently does not turn black. With both these acids ferric chloride produces a red coloration that is destroyed by the addition of a mineral acid; on boiling also (without the addition of a mineral acid), the red-coloured solution loses colour and deposits a rust-coloured precipitate. Butyric acid is recognisable by its odour, like that of rancid butter, and by forming non-crystallisable, volatile salts with the alkalis; with the alkaline earths, as barium, its salts crystallise in the form of prisms which form groups, or clumps. Propionic acid forms a silver salt on the addition of silver nitrate which, when dissolved in boiling water, is partially reduced; but it gives no coloration with ferric chloride.

All these fatty acids have distinctive, penetrating odours; that of formic acid is peculiarly pungent, like that of sulphur dioxide. Their boiling-points differ: formic acid boils at 100° C.; acetic acid at 118° C.; propionic acid at 141° C.; butyric acid at 163° C. Except formic and acetic acids, all the fatty acids, on the addition of calcium chloride, are separated from their solutions in water and form an oily layer on its surface.

LACTIC ACID. $C_3H_5O_3$.

Lactic acid or α -hydroxypropionic acid is miscible with water, alcohol, and ether, in all proportions. When heated with dilute sulphuric acid it is decomposed into aldehyde and formic acid. Lactic acid forms metallic and ethereal salts. Sarcolactic acid has the same constitution as lactic acid, but differs from it in being optically active; free sarcolactic acid is feebly dextro-rotatory; its salts are feebly lævo-rotatory.

Under ordinary conditions lactic acid is not present in urine. It appears in consequence of imperfect oxidation, such as is produced in animals by placing them in a closed chamber of limited air-capacity, and in man by carbon-monoxide poisoning, or by an epileptic seizure. Araki¹ found, in cases of acute poisoning in which lactic acid was excreted, that loss of glycogen occurred, the imperfect oxidation of which furnished the lactic acid. In some severe diseases of the liver the organ becomes incapable of dehydrating into urea, the ammonium lactate derived from the muscles; hence the acid appears in the urine. This occurs in acute phosphorus poisoning, and in acute atrophy of the liver. Lactic acid has been found in the urine after prolonged muscular exercise, the oxidising power of the liver being unable to deal with the excess of the acid temporarily produced. In addition to the conditions already named, lactic acid has been found in cirrhosis of the liver, in pernicious anæmia, in poisoning by morphine, cocain, arsenic, and amyl nitrite, and also in various diseases immediately before death.

Separation.—Lactic acid may be extracted from urine, after free acidulation with sulphuric or phosphoric acid, by large amounts of ether. This is most conveniently done with the aid of a fat-extraction apparatus, in which, by means of heat, a supply of ether is made continuously to pass through the urine for twelve or twenty-four hours, and thus thoroughly to exhaust it. The ethereal extract is evaporated to dryness, and the residue, dissolved in water, is boiled with excess of zinc carbonate and filtered. The filtrate, concentrated

¹ *Zeitschr. f. physiol. Chem.*, 1891.

on the water-bath, is allowed to stand till crystals form; this may be aided by the addition of alcohol. After separation the crystals are washed with alcohol and dried in a porcelain capsule at 100° C. They are then covered with a little nitric acid and gently heated till the acid is driven off, when the residue is carefully ignited and the resulting zinc oxide is weighed; dry zinc lactate contains 33.42 per cent. of zinc oxide.

The colour-tests for lactic acid are unreliable, as some of the volatile fatty acids, phosphates, alcohol, and sugar may give the same reactions.

OXALIC ACID. $C_2H_2O_4$.

Oxalic acid is probably a normal and constant constituent of human urine, but it is not always to be found. On a mixed diet Salkowski¹ found 0.128 grm. in the twenty-four hours, which exceeds the amounts found by previous investigators. Its presence is to be attributed to extrinsic and intrinsic sources; to oxalic acid-yielding substances ingested as food, and to synthetic production in the organism. Pierallini² found that the normal average excretion of oxalic acid in three women ranged from 0 to 6 mgs. a day; after administering 2 to 15 cgs. of oxalic acid, the excretion rose to 0.03 grm. A like result followed the ingestion of spinach and tea, both of which contain oxalic acid. Faust³ found 92 to 95 per cent. of administered oxalic acid in the urine. Lommel,⁴ whilst admitting that food rich in nucleins, such as the thymus of the calf, and also gelatine, increases the output of oxalic acid, states that neither carbohydrates nor proteids produce any increase. He finds that most of the oxalic acid received in food is decomposed in the organism, as only a fraction of it appears in the urine and fæces; by far the greatest amount contained in the urine is produced in the organism. Salkowski rejects proteids as a source of oxalic acid, and thinks that if not actually derived from uric acid itself it is from nucleins, out of which it, or its precursor oxaluric acid, is probably formed in the liver. Lüthje⁵ gave thymus and nucleins to a convalescent without obtaining any definite result; neither was he more successful with grape-sugar, thus agreeing with the negative results obtained by Mills.⁶ Lüthje concludes that oxalic acid is not derived from nuclein-containing foods, but that it is formed in the organism. Mayer⁷ found that the administration of 40 grms. of grape sugar to a

¹ *Berlin. klin. Wochenschr.*, 1900.

² *Virchow's Arch.*, 1900.

³ *Arch. f. exp. Pathol.*, 1900.

⁴ *Deutsches Arch. f. klin. Med.*, 1889.

⁵ *Zeitschr. f. klin. Med.*, 1898, 1900.

⁶ *Virchow's Arch.*, 1885.

⁷ *Congress f. innere Med.*, 1901.

rabbit increased the excretion of oxalic acid in the urine. Mayer¹ has further shown that the liver is able to oxidise glycuronic acid to oxalic acid. Hildebrandt² also found that sugar caused more oxalic acid to appear in the urine, but that when half a gramme of oxalic acid was given to a rabbit only 7.2 mgs. appeared in the urine; from this he infers that the oxalic acid which is ingested is decomposed by the putrefactive processes which take place in the intestine. The statement formerly accepted that increased excretion of oxalic acid usually accompanies diabetes is doubtful; in seven cases of mild diabetes, the patients' diet being varied, Mohr and Salomon³ found no increase; when it occurs Klemperer attributes it to the large amount of animal food that is eaten by diabetics. A condition described as idiopathic oxaluria is also doubtful, although in clinical practice cases are met with in which excessive excretion of oxalic acid occurs, associated with pain and heaviness in the lumbar region, hypochondriasis, and emaciation, without any ascertainable cause. The whole chain of symptoms is probably due to perverted metabolism, which may or may not be attended by increased excretion of oxalic acid. The appearance of crystals of calcium oxalate in the urine does not necessarily indicate excessive excretion of the acid; in many of these cases, however, an actual excess has been determined. Neidert⁴ found 0.5 gram. to the litre of urine in a case of this kind.

Separation.—Oxalic acid may be separated by Salkowski's⁵ improved process: 300 c.c. of urine are evaporated down to two-thirds; when cold, 20 c.c. of hydrochloric acid are added, and the urine is then extracted with 200 c.c. of a mixture of ether and alcohol (10:1). After allowing the ether to evaporate 10–15 c.c. of water are added to the alcohol, and the whole is evaporated on the water-bath to about 20 c.c., when some resin-like masses separate. The liquid is then filtered, and the filtrate is made slightly alkaline with ammonia; 1 or 2 c.c. of a 10 per cent. solution of calcium chloride are added, and then acetic acid to a distinct acid reaction, so that all the calcium phosphate that is thrown down is dissolved. After standing twenty-four hours the deposit of calcium oxalate is filtered off, washed, dried, and ignited, and calculated as calcium oxide: 100 parts of CaO = 225 parts of crystallised oxalic acid.

Oxaluric acid ($C_3H_4N_2O_4$) is a monureide, that is, a urea in which part of the hydrogen is replaced by a dyad acid-radicle. It was first obtained from urine by Schunck⁶ who expressed the opinion that it is a constituent of the normal secretion. This has been confirmed

¹ *Zeitschr. f. klin. Med.*, 1902.

² *Zeitschr. f. physiol. Chem.*, 1902.

³ *Deutsches Arch. f. klin. Med.*, 1901.

⁴ *Münchener med. Wochenschr.*, 1890.

⁵ *Zeitschr. f. physiol. Chem.*, 1900.

⁶ *Proc. Roy. Soc.*, 1867.

by, amongst others, Salkowski, who believes that preformed oxaluric acid is present in normal urine in greater amount than is generally supposed.

Chondroitin-sulphuric acid, $C_{18}H_{26}NO_{13}-SO_3\cdot HO$. This mono-basic acid, which in small amount is present in normal urine, is derived from cartilaginous tissues, and from the intima of the aorta; pathologically, it has been obtained from amyloid livers. It has a strong acid reaction and is very soluble in water, the solution being lævo-rotatory. It forms combinations with metals, and with albumin—chondro-albumin. The proteid combinations are soluble in strong acids, and in alkalies, and from alkaline and saline solutions they are precipitated by acids; they are also partially precipitated by magnesium sulphate, and almost entirely by ammonium sulphate; they give the colour reactions of proteids.

Separation.—Mörner¹ dialyses about one litre of urine (which should contain a little albumin) in running water until it is almost free from chlorides; after filtration it is treated with from 0.1 to 0.2 per cent. of acetic acid, and to promote precipitation of the combination of chondroitin-sulphuric acid and albumin the urine is well shaken with chloroform. It is allowed to stand for one or two days, when the precipitate, after being filtered off, is dissolved in dilute ammonia and tested for sulphuric acid by adding a little barium chloride and from 2 to 5 per cent. of hydrochloric acid, and warming the solution on the water-bath for a few hours; the sulphuric acid which is set free is precipitated as barium sulphate. The presence of the other component—chondrosin—is shown by its power of reducing Fehling's solution.

Nucleinic acid is a combination of phosphoric acid, xanthin bases, and non-nitrogenous matter; it contains nearly 10 per cent. of phosphorus. It has an acid reaction and, like chondroitin-sulphuric acid, forms combinations with albumin—nucleo-albumin—which are also precipitated from acid solution. Mörner² finds nucleinic acid to be present in very small amount in normal urine.

Separation is effected by the same process as that described for separating chondroitin-sulphuric acid, the distinction between the two acids being made by determining the presence of phosphoric acid and of xanthin bases which are indicative of nucleinic acid, just as the presence of sulphuric acid and of chondrosin are indicative of chondroitin-sulphuric acid.

It is to be noted that, in acid solution, both these acids are precipitants of albumin.

Several other organic acids, which are of but little practical

¹ *Skand. Arch.*, 1895.

² *Loc. cit.*

moment, have been found in human urine; amongst them are succinic and glycerinphosphoric acids.

Succinic acid ($C_4H_6O_4$) has been found in human urine in very small amount. Meissner¹ places it among the constituents of normal urine; this is not generally accepted. Salkowski² failed to obtain any evidence of the presence of succinic acid in urine.

Glycerinphosphoric acid ($C_3H_7O_3 \cdot PO_2OH$) is the chief constituent of lecithin, and some investigators have assumed that the acid does not appear as such in the urine, but as lecithin. Glycerinphosphoric acid is one of the substances which, along with nucleinic acid in proteid combination, represents the organically combined phosphoric acid of urine. In some pathological conditions comprising certain forms of hepatic disease, especially when accompanied by fatty changes, in jaundice due to complete blocking of the bile ducts, in some fevers, and in phthisis, it has been found in the urine in increased amount. In normal urine it does not exceed 1 per cent. of the inorganic phosphoric acid, and under pathological conditions it does not exceed 4 per cent.

Sulphocyanogen ($HS \cdot CN$) in small amount is present in human urine—from 3 mgrms. (Bruylants³) to 1 decigram. (Munk⁴) of potassium sulphocyanide have been found in the litre. The sulphur which enters into the composition of this salt constitutes one of the chief sources of the neutral sulphur of urine. In rabbits the subcutaneous injection of sodium sulphocyanide caused a considerable increase in the excretion of sulphur and of nitrogen (Treupel and Edinger⁵); but Pollak⁶ found that when the same salt was given to human beings, either subcutaneously or by the mouth, it could all be recovered from the urine, showing that the liver has no power to decompose it. The inhalation of carbon bisulphide enormously increases the excretion of potassium sulphocyanide.

The *detection* of the sulphocyanide is best accomplished by Munk's method: 200 c.c. of urine are acidified with nitric acid and then precipitated with silver nitrate; the precipitate is decomposed by sulphuretted hydrogen and the filtrate is distilled. The distillate is treated with a little solution of ferrous sulphate which contains a little ferric salt, and is then made alkaline with potash; the addition of a little hydrochloric acid develops a blue colour, due to the formation of Prussian-blue.

¹ *Untersuch. über d. Entstehung d. Hippursäure*, 1866.

² Pflüger's *Arch.*, 1871.

³ *Bull. d. l'Acad. de méd. Belg.*, 1888.

⁴ *Deutsche med. Wochenschr.*, 1876.

⁵ *Ibid.*, 1900.

⁶ Hofmeister's *Beiträge z. chem. Physiol.*, 1902.

HIPPURIC ACID. $C_7H_6O_2$

Hippuric acid, or benzoyl-glycin, is produced in the organism by the conjugation of dehydrated benzoic acid and glycin. In human urine the daily excretion varies from 0.5 to 1 grm., but it is very exceptional for hippuric acid spontaneously to form a deposit; in the urine of herbivora it is much more abundant. Hippuric acid crystallises in fine needles, often grouped together, or in larger four-sided prisms, the sides being bevelled at each end of the prisms to a pyramidal form. It is not very soluble in cold water, nor in ether, but is much more soluble in hot water and in alcohol. In dilute aqueous solution hippuric acid reddens litmus-paper, and 1 part in 55,000 of water gives the reaction of a free acid with Congo-red; urine does not change the colour of Congo-red, which shows that the hippuric acid that is present is in combination with bases. It is a monobasic acid, and its salts, except its combinations with iron, are much more soluble than is the free acid. When strongly heated it yields benzoic acid; when heated with the mineral acids it is split into benzoic acid and glycin.

In human beings hippuric acid is chiefly derived from the aromatic products of proteid decomposition, principally of food proteids, and, to a less extent, of tissue-proteids. Jolles¹ states that glycin and the amido-acids occur as intermediate products in the ordinary course of the oxidation of proteids into urea. In addition to this origin, the introduction into the system of benzoic acid, or of its potential components—such as quinic acid, which is reduced in the organism to benzoic acid (Weiss²)—causes an increased excretion of hippuric acid, and as might be inferred from the amount of hippuric acid in the urine of herbivora, the ingestion of large quantities of vegetable food, and also fruits, such as bilberries and stone fruit, produces a like result. It is usually assumed that in mammals the conjugation of benzoic acid and glycin takes place in the kidneys. This was demonstrated by Schmiedeberg and Bunge³ who produced hippuric acid by passing benzoic acid and glycin through recently excised kidneys. Some investigations made by Sertoli⁴ show that there is no diminution in the excretion of hippuric acid in cases of Bright's disease; from this he concludes that the kidneys are not the organs in which the synthetic formation of hippuric acid occurs, and that no conclusions can be drawn from the amount of hippuric acid excreted as to the intensity of any

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1900.

² *Zeitschr. f. physiol. Chem.*, 1898.

³ *Arch. f. exp. Path.*, 1876.

⁴ *Gazz. degli ospedali*, 1898.

pathological changes in the kidneys. Sertoli states that in Bright's disease, the daily excretion of hippuric acid ranges from 1.224 grms. to 0.1115 gram. On the other hand, Abelous and Ribaut¹ demonstrated experimentally that the kidneys contain a soluble ferment which, *in vitro*, forms hippuric acid from benzylalcohol and glycin. Lewin² found that persons living chiefly on milk diet excreted from 0.1 to 0.3 gram. of hippuric acid daily; in gout and diabetes he found no alteration; but in febrile diseases and in Bright's disease more was excreted. In fever other observers have found less than normal; in erysipelas Blumenthal found a considerable increase, the highest amounting to 1.1 grms.

Reactions.—When gently heated it does not at once sublime like benzoic acid, but it melts, and on cooling again becomes solid; at a higher temperature it gives off benzoic acid and develops the odour of hay, which subsequently changes to that of hydrocyanic acid. If a little solution of neutral ferric acetate is added to a solution of hippuric acid a precipitate is formed.

The *estimation* of hippuric acid may be made by first slightly alkalisng the urine and then evaporating it nearly to dryness. The deposit is extracted with absolute alcohol, the extract is evaporated, and the residue is dissolved in water, acidulated with sulphuric acid, and extracted four or five times with acetic ether. The ethereal extract is repeatedly washed with water, and then, at a moderate heat, is evaporated to dryness. The residue, after being well shaken with petroleum ether (in which hippuric acid is insoluble) in order to remove benzoic acid, oxyacids, fat, phenol, &c., is dissolved in a little warm water and, at 60° C., is evaporated down to crystallisation. The crystals are collected on a small tared filter and weighed (Bunge and Schmiedeberg³).

Blumenthal⁴ substitutes the following process as being more suitable for the estimation of the small amount of hippuric acid present in human urine: 300 c.c. of urine, made feebly alkaline with soda, are evaporated to dryness on the water-bath. The residue is twice extracted with successive amounts (150 c.c.) of 90 per cent. alcohol, with the aid of heat. After filtration, the extracts are evaporated to a syrup, which is dissolved in 50 c.c. of water containing 10 c.c. of 25 per cent. hydrochloric acid; this is well shaken four times, each time with 150 c.c. ether-alcohol (10:1). After the ethereal extracts have been washed with about 75 c.c. of water they are evaporated, and the amount of nitrogen contained in the

¹ *Comptes Rend. Soc. Biol.*, 1900.

³ *Arch. f. exp. Path.*, 1876.

² *Zeitschr. f. klin. Med.*, 1901.

⁴ *Zeitschr. f. klin. Med.*, 1900.

residue is estimated by Kjeldahl's process. Each cubic centimetre of N/10 sulphuric acid corresponds to 17.9 mgms. of hippuric acid.

HOMOGENTISIC ACID. $C_8H_8O_4$.

Homogentisic acid is chiefly of clinical interest as being the substance to which the colour and the reducing property of the urine in alkaptonuria are due; in this condition 2.26 grms. (Mittlebach¹) to 3.24 grms. (Meyer²) may be excreted daily. The way in which homogentisic acid is produced in the organism is obscure. Baumann states that it is derived from the proteid molecule, and regards tyrosin as the parent substance; he believes that homogentisic acid is formed by specific micro-organisms in the small intestine. Wolkow and Baumann,³ Embden,⁴ and Stier⁵ found that when tyrosin is given by the mouth to a patient with alkaptonuria the amount of homogentisic acid in the urine is increased. Mittlebach also found that tyrosin produces this effect when given in repeated small doses. Meyer found that proteid food, *e.g.*, plasmon, increased the output of homogentisic acid to 5.27 grms. daily. Falta and Langstein,⁶ in a case of alkaptonuria, found that the tyrosin derived from the various proteids was not sufficient to account for the homogentisic acid which was daily excreted. When phenylalanine was administered a large proportion appeared in the urine as homogentisic acid. Phenylalanine, or β -phenyl- α -amidopropionic acid, is an integral constituent of the proteid molecule, and is related to tyrosin. Gonnermann⁷ states that the darkening of certain vegetable juices is due to oxidation (by enzymes) of derivation-products of tyrosin, and that by the action of the hydrolysing-enzyme *tyrosinase*, homogentisic acid is produced from tyrosin. On the other hand, as observed by Huppert,⁸ the hydroxyl and the α -amido-propionic acid in tyrosin occupy the para, or 1:4 position, and the transformation of tyrosin into homogentisic acid in alkaptonuria implies an interchange of the hydroxyl group within the organism. In some of the cases of alkaptonuria homogentisic acid was experimentally administered, with the result that a large proportion of it, as much as 75 per cent., was excreted in the urine; in the healthy individual this is not the case. Embden⁹ himself took 4 grms. of homogentisic acid, but no trace of it could

¹ *Deutsch. Arch. f. klin. Med.*, 1901.

² *Ibid.* 1901.

³ *Zeitschr. f. physiol. Chem.*, 1891.

⁴ *Ibid.* 1892.

⁵ *Berl. klin. Wochenschr.*, 1898.

⁶ *Zeitschr. f. physiol. Chem.*, 1903.

⁷ *Pflüger's Archiv*, 1900.

⁸ *Zeitschr. f. physiol. Chem.*, 1897.

⁹ *Zeitschr. f. physiol. Chem.*, 1894.

be found in the urine, and the uric acid output was unaltered; probably the homogentisic acid was destroyed in the tissues. In some cases of alkaptonuria a diminution in the excretion of uric acid has been observed, in others it remained unchanged.

Separation.—Wolkow and Baumann¹ separate homogentisic acid from urine by acidifying with sulphuric acid and extracting with ether; after evaporation the residue of the ethereal extract is dissolved in warm water, and the aqueous solution, heated nearly to boiling, is treated with a concentrated solution of lead acetate; on cooling, the lead homogentisate crystallises out. Lead homogentisate has a melting-point of 214° C.

Garrod² simplifies this method by directly treating the urine with lead acetate. To every 100 c.c. of the urine, heated nearly to the boiling-point, 5 or 6 grms. of solid, neutral lead acetate are added; the urine is then left to stand in a cool place for twenty-four hours, when minute acicular crystals of lead homogentisate are deposited; from these the acid may be liberated by sulphuretted hydrogen. When homogentisic acid is dissolved in water the solution turns brown on the addition of an alkali, and reduces Fehling's fluid. With ferric chloride a blue colour is produced.

Orton and Garrod³ obtained homogentisic acid from alkapton urine by means of benzoylation. A litre of the urine is shaken with 15 c.c. of benzoyl chloride, and with 150 c.c. of a 10 per cent. solution of sodium hydrate, gradually added, until the odour of the benzoyl chloride disappears. The ester which falls is washed with water and, when dry, is extracted with boiling alcohol. The hot alcoholic extract is filtered into an excess of water, and the precipitate that forms is purified by re-crystallisation out of alcohol; it consists of dibenzoyl-homogentisic acid-amide, which has a melting-point of 204° C. Homogentisic acid is split from the amide by treatment with nitroso-nitric acid. Meyer⁴ obtained homogentisic acid directly from the urine as an ethyl-ester. The acidulated urine is extracted with a mixture of ether-alcohol; the extract is evaporated to a syrup, alcohol is added, and the whole is boiled for a long time on the water-bath. The resulting syrup, rubbed up with water, throws down a crystalline ester which is dried on a porous plate; its melting-point is 120° C.

Uroleucic acid ($C_9H_{10}O_5$) was discovered by Kirk⁵ in the urine from a patient with alkaptonuria. In the impure product from the urine of the same patient Huppert⁶ found both uroleucic and homo-

¹ *Zeitschr. f. Physiol. Chem.*, 1891.

² *Journ. of Physiol.*, 1898.

³ *Journ. of Physiol.*, 1901.

⁴ *Deutsches Arch. f. klin. Med.*, 1901.

⁵ *Journ. of Physiol.*, 1889.

⁶ *Zeitschr. f. physiol. Chem.*, 1897.

gentisic acids. Uroleucic acid has not been found in any other cases of alkaptonuria.

Uroleucic acid is a crystalline monobasic acid, with a melting-point of 133.3°C . It is soluble in alcohol and in ether, but is not so soluble in water. A quarter per cent. solution gives a deep, reddish-brown colour with alkalies; a transient green with 1:40 ferric chloride; no precipitate with neutral lead acetate; a white precipitate with basic lead acetate, which becomes violet on exposure to air. Uroleucic acid reduces Fehling's solution, and in 0.5 per cent. solution it reduces an alkaline bismuth solution; urine containing uroleucic acid, however, does not reduce bismuth, because the percentage of the acid that is present is insufficient.

Some of the hydroxy acids of the aromatic group, which have also been found in human urine, are: **paraoxyphenylacetic acid** ($\text{C}_8\text{H}_8\text{O}_3$), **hydroparacoumaric acid** ($\text{C}_9\text{H}_{10}\text{O}_3$), and **oxymandelic acid** ($\text{C}_8\text{H}_8\text{O}_4$). The first two were found by Baumann¹ in normal urine; they are produced in the course of proteid decomposition in the intestines, and therefore are developed on parallel lines with indol. A small proportion of these acids may be present in the urine as conjugated acids, which are excreted with the other ether-sulphates. Oxymandelic acid has been found in the urine in cases of acute yellow atrophy of the liver (Schultzen and Riess²), and in acute phosphorus poisoning (Baumann³).

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1880.

² *Ann. d. Charité Krankenh.*, 1869.

³ *Zeitschr. f. physiol. Chem.*, 1882.

AMIDO AND AROMATIC ADCIS.

CREATIN. $C_4H_9N_3O_2$.

CREATIN, or methylguanidinacetic acid, has been found along with creatinin in human urine, but only in very small amount. The facility with which creatin and creatinin are reciprocally converted, the one into the other, by the processes used for their extraction from urine, conduces to error of interpretation of the results obtained, and on this ground the alleged presence of creatin in urine has been disputed.

CREATININ. $C_4H_7N_3O$.

Creatinin, or glycolylmethylguanidin, is a dehydrated form of creatin, to which it bears the same relation that parabanic acid does to oxaluric acid. Creatinin has been successively regarded as a decomposition product of proteids in general, as an antecedent of urea, and as a specific product of muscle-metabolism. It is present in normal urine, averaging about one gramme in the twenty-four hours; when large amounts of animal food are eaten the excretion of creatinin is increased. Children at the breast excrete no creatinin, but they do so if given other food than milk. In the case of a healthy person who successively lived on (a) animal food, and (b) creatinin-free diet, which respectively contained about equal amounts of nitrogen and were of the same calorific value, Macleod¹ found that when on (a) 2.098 grms. of creatinin were excreted daily, when on (b) only 1.064 grms. By restricting a patient with hypoleucocytosis to a creatinin-free diet, he found the average amount of endogenous creatinin excreted in urine to be only 0.332 gm., and in a case of leucocythæmia 0.530 gm. Tedeschi² found that diseases which are attended by excessive muscular metabolism are characterised by increased excretion of creatinin in the urine both absolutely and in relation to the total nitrogen.

Creatinin is soluble in water 1 : 12; less so in alcohol, and scarcely at all in ether. It crystallises in prisms and in the whetstone form, resembling the crystals of uric acid. It is a basic substance, although when pure its reaction is neutral. It combines with acids, and also

¹ *Journ. of Physiol.*, 1901.

² *Riv. Veneta d. scien. Med.*, 1901.

forms double salts with metals, some of which are but slightly soluble in water, a fact that is taken advantage of in order to precipitate creatinin from aqueous solution. When boiled with Fehling's solution creatinin reduces the copper salt, but at the same time prevents the precipitation of the oxide; the reduction being simply indicated by the solution changing from blue to yellow. [See the action of glucose on Fehling's solution.]

Reactions.—*Weyl's test*.¹—The presence of creatinin in urine may be detected by the addition of a few drops of a freshly-prepared, very weak solution of sodium nitroprusside and a few drops of a solution of caustic soda; a red colour is produced which quickly fades away. On the subsequent addition of excess of acetic acid, with the application of heat, the solution turns greenish and afterwards blue (Salkowski).² Acetone gives a red colour with Weyl's test, but not the subsequent blue. If ammonia be substituted for soda, no red colour is produced by creatinin, which also distinguishes it from acetone.

Jaffe's test.³—A little solution of picric acid and a few drops of a dilute solution of caustic soda produces a deep red colour with very dilute solutions of creatinin—1 : 3000. Acetone gives an orange-red with the same reagents.

The *estimation* of creatinin in urine may be made by G. S. Johnson's⁴ modification of Maly's process. To a measured amount of urine one-twentieth its volume of a saturated solution of sodium acetate and one-fourth its volume of a saturated solution of mercuric chloride are added. The solution is then immediately filtered and the filtrate is allowed to stand for forty-eight hours. The compound of creatinin and mercury, which has then fallen, is separated and after being distributed in water is decomposed by sulphuretted hydrogen. After filtration from the deposit of mercurous sulphide, the filtrate, which consists of a solution of creatinin hydrochloride, is evaporated down *in vacuo*; the residue, dissolved in fifteen times its weight of water, is treated with excess of recently precipitated lead hydrate, and is evaporated *in vacuo* over sulphuric acid, after which it deposits creatinin in the crystalline form. Allen⁵ prefers to deal with the mercury salt by Kjeldahl's process; the amount of creatinin being deduced from that of the ammonia obtained.

Creatinin also forms salts with zinc chloride and silver nitrate. The zinc salt may be obtained by adding a saturated alcoholic solution of zinc chloride to an alcoholic extract of urine which has been

¹ *Berichte der deutsch. chem. Gesellsch.*, 1878.

² *Zeitschr. f. physiol. Chem.*, 1880.

³ *Zeitschr. f. physiol. Chem.*, 1886.

⁴ *Proc. Roy. Soc. Lond.*, 1888, 1892.

⁵ *Chemistry of Urine*, 1895.

concentrated by evaporation; clumps of small prisms crystallise out, which are insoluble in alcohol.

LEUCIN. $C_6H_{13}NO_2$.

Leucin, or α -amido-caproic acid, is a decomposition product of proteid substances and of gelatine. It occurs in isomeric forms, one of which is lævo-rotatory; others are dextro-rotatory; the isomer derived from the tissues is dextro-rotatory. When found in urine leucin appears as small, yellow, fatty-looking balls, which feel greasy to the touch. Under the microscope radial markings may be seen, and, sometimes, concentric lines. Leucin is rather soluble in cold water—1 : 27—and in alcohol, but not in ether; it readily dissolves in alkalies and acids. When present in the urine it is usually associated with tyrosin and occurs in acute atrophy of the liver and, occasionally, in acute phosphorus poisoning; it has also been found in severe cases of typhoid fever and small-pox, in pernicious anæmia, and in erysipelas, without any special affection of the liver. A very exceptional instance is recorded by Smith¹ in which the urine of a healthy girl, aged 23, deposited a considerable layer of white sediment like an ordinary phosphate deposit. Under the microscope this was seen to consist of spheroid and discoid bodies, which yielded many of the reactions of leucin, the inference being that it included at least one isomer of leucin.

Tests.—The chemical tests for leucin are not applicable unless the leucin is in a pure state, in which it is with difficulty obtained from urine. When heated on platinum foil, it sublimes at 170° C. without melting, in white flocculent clouds, and gives off a peculiar odour. Leucin, in small amount, is best recognised by its microscopical appearance and its solubilities (*vide* Urinary deposits).

TYROSIN. $C_9H_{11}NO_3$.

Tyrosin, or oxyphenyl amido-propionic acid, is also a decomposition product of proteids, but not of gelatine. When present in urine it is usually in association with leucin; occasionally it occurs alone. Neither leucin nor tyrosin are present in normal urine. As met with in urine, tyrosin crystallises in the form of fine needles which tend to arrange themselves in sheaves, or bundles, of a yellowish or greenish colour; pure tyrosin is colourless. Tyrosin is very much less soluble than leucin in cold water—1 : 2000; it is soluble in alkalies and acids, and is insoluble in absolute alcohol and ether. When considerable amounts of leucin and tyrosin are present in

¹ *The Practitioner*, 1903.

urine crystals of tyrosin may be spontaneously deposited, whilst the leucin remains in solution.

Tests.—If, to a little tyrosin dissolved with the aid of heat in a few drops of water, Millon's reagent (acid nitrate of mercury) is added, a purplish colour is produced and, after further boiling, a red precipitate falls unless the amount of tyrosin is very small (Hoffmann). On adding to 2 c.c. of sulphuric acid in a test-tube four or five drops of a 50 per cent. solution of aldehyde in alcohol, and then a few drops of a solution of tyrosin, a carmine red colour is produced; this gives a broad absorption band in the green, which covers the green and most of the yellow of the spectrum. This test will react to the one-hundredth of a milligramme of tyrosin (Denigès¹).

The *separation* of leucin and tyrosin from urine is accomplished by precipitating albumin-free urine with basic lead acetate; the urine, filtered from the precipitate, is freed by sulphuretted hydrogen from the lead which is in solution, and is then evaporated to a syrup. This is repeatedly extracted with small quantities of absolute alcohol in order to remove the urea. The residue is treated with hot dilute alcohol to which some ammonia has been added, and, after filtration, the filtrate is evaporated to a small volume and is then left for the tyrosin to crystallise out. After separation of the tyrosin the liquid is further concentrated in order to obtain the leucin.

Habermann and Ehrenfeld² recommend the separation of leucin from tyrosin by boiling them in glacial acetic acid in which the leucin is readily dissolved, whilst the tyrosin remains untouched. The impure leucin, which is in solution in the acetic acid, is purified by boiling the solution for a few minutes with animal charcoal, filtering and evaporating the acetic acid; the residue is dissolved in 95 per cent. boiling alcohol, from which it is re-crystallised.

CYSTIN. $(C_3H_6NSO_2)_2$.

Cystin, amido-sulpholactic acid, if present in normal urine at all, it is only as a trace. When deposited from urine cystin forms a greyish sediment, and the urine on keeping gives off sulphuretted hydrogen, which, however, may be evolved from urine in the absence of cystin. It is insoluble in water, acetic acid, alcohol, and ether; it is soluble in the mineral acids, ammonia and the fixed alkalies. Its ready solubility in ammonia causes a deposit of cystin to disappear when the urine has passed a certain stage of alkaline fermentation with the evolution of free ammonia; in the earlier stages, when all the ammonia is combined as carbonate, the cystin deposit is

¹ *Compt. Rendus*, 1900.

² *Zeitschr. f. physiol. Chem.*, 1902.

unaffected, as it is insoluble in ammonium carbonate. Urine which contains cystin in solution is usually of a pale yellow colour, often neutral or slightly acid in reaction; sometimes it is feebly alkaline.

Cystin is a cleavage product of proteid matter which can be obtained by hydrolysis of keratin (Mörner¹). By the action of bromine-water Friedmann² oxidised cystin to cysteinic acid, and by heating cysteinic acid with baryta-water he obtained a small amount of serin, or silk-glue, a substance which is obtained from raw silk. By hydrolysis of serin Fischer³ obtained a peptone-like body which, by the action of trypsin, yielded a large quantity of tyrosin. Friedmann believes that proteid substances, which contain no lightly-bound sulphur and which consequently are not cystin, contain serin, and that cystin and serin may replace each other in the animal organism.

The presence of cystin in urine is due to a peculiar perversion of metabolism which has a tendency to run in families, several members of such families being the subjects of cystinuria. Cohn⁴ records the case of a woman and six of her children who were thus affected. Cystinuria usually occurs without any recognisable deviation from health. Pfeiffer⁵ relates that four sisters, whose father was gouty, all had cystinuria, and except for the urinary trouble suffered no inconvenience; in one of these cases, 0.8672 grm. of cystin was excreted in the twenty-four hours. Cystinuria may occur in the course of an acute disease; it has been found in acute rheumatism and also in acute phosphorus poisoning. Many observers hold that cystinuria is due to the action of micro-organisms. Delépine⁶ states that in certain urines a compound exists which, by fermentation, yields cystin, and that such fermentation may be set up by an organism of considerable size, probably one of the *blastomycetes*. Brieger⁷ believes that in cystinuria certain putrefactive processes in the intestines are set up by bacteria, and consequently that diamines are formed and may be detected both in the fæces and in the urine. In a few instances diamines have been found; in one case of cystinuria Baumann and Udránszky⁸ constantly found cadaverin and putrescin both in the urine and in the fæces. Simon⁹ found that cadaverin was always present, and that putrescin was always absent in both the urine and the fæces. On the other hand, out of thirty analyses made by Cammidge and Garrod¹⁰ in a case

¹ *Zeitschr. f. physiol. Chem.*, 1899.

² Hofmeister's *Beiträge z. chem. Physiol.*, 1902.

³ *Vortrag*, Karlsbad, 1902.

⁴ *Berliner klin. Wochenschr.*, 1899.

⁵ *Centralbl. f. d. Krankh. d. Harn.-u. Sex.-Org.*, 1897.

⁶ *Proc. Roy. Soc.*, 1890.

⁷ *Berliner klin. Wochenschr.*, 1889.

⁸ *Zeitschr. f. physiol. Chem.*, 1889, 1891.

⁹ *American Journ. Med. Sc.*, 1900. ¹⁰ *Journ. of Path. and Bacteriol.*, 1900.

of cystinuria, on two occasions only was cadaverin found in the urine; and out of six analyses of the fæces from the same case putrescin was only found once; they also state that the occurrence of the diamine in the urine did not correspond with the period of excretion of a similar product in the fæces. In Pfeiffer's cases, and also in those reported by Cohn, no diamines were found either in the urine or in the fæces. In the urine of a woman who had suffered from cystinuria for three years, Moreigne¹ found absolute diminution of nitrogen-excretion with relative diminution of urea; the oxidised sulphur was diminished with relative increase in the incompletely oxidised sulphur; the phosphoric acid was relatively diminished, with increase of extractives and the presence of leucin, tyrosin, and diamines. He sees no causal relation, however, between the occurrence of the diamines and the formation of cystin, and is opposed to the view that the latter is caused by intestinal fermentation, as no cystin appeared in the fæces, there was no excess of indican, and treatment by intestinal antiseptics produced no effect on the formation of the cystin. Moreigne regards cystinuria as an indication of delayed metabolism, with imperfect oxidation-processes in the body. Many observers have found, in cystinuria, that the proportion of ether-sulphates to simple sulphates is very much increased; this is not necessarily the result of increase in the ether-sulphates due to intestinal putrefactive processes, but possibly of diminution of the preformed sulphates proportionally to the unoxidised sulphur which is excreted in the cystin.

Reactions.—When urine contains much cystin in solution, it may be precipitated by freely acidulating it with acetic acid, or, as recommended by Delépine, it may be allowed to undergo spontaneous acid fermentation. If only a trace of cystin be present it may be isolated by benzylation, as adopted by Goldmann and Baumann²:—to 200 c.c. of the urine, 70 c.c. of solution of caustic soda (S.G. 1.12) and 10 c.c. of benzoyl chloride are added, and the whole is well shaken until the odour of benzoyl chloride disappears. The ester which is formed contains the benzoyl combinations of the normal urinary carbohydrates and a part of any diamine combinations which may be present, along with phosphates; it is filtered off and the filtrate, well acidulated with sulphuric acid, is extracted with a mixture of ether and alcohol. The ethereal extract is evaporated and the residue is heated over a water-bath for some hours with caustic soda and lead acetate. The lead sulphide

¹ *Arch. de Méd. expériment.*, 1899.

² *Zeitschr. f. physiol. Chem.*, 1888.

which results is equivalent to about two-thirds of the cystin isolated. To obtain cystin, which is in solution in urine, in a crystalline form, the urine is either strongly acidulated with acetic acid and allowed to stand for twenty-four hours, or it is allowed to undergo spontaneous acid fermentation. The precipitate which falls is digested with hydrochloric acid by which the cystin and any calcium oxalate is dissolved; the uric acid which has come down remains untouched. The solution is filtered and then supersaturated with ammonium carbonate and the precipitated cystin is treated with ammonia, by which it (but not any calcium oxalate) is dissolved. After again filtering, the cystin is finally thrown down by the addition of acetic acid, when its crystals may be recognised by the microscope. They may further be tested by being boiled with soda or potash solution; on the addition of lead acetate lead sulphide is formed. Cystin is precipitated from its solution in dilute sulphuric acid by mercuric sulphate, and from the white precipitate which forms it can be recovered by treatment with sulphuretted hydrogen; after evaporation of the filtrate, the cystin can be extracted by ammonia (Riza¹). Cystin is precipitated by mercuric chloride, but the salt is reduced.

Carbamic acid (CH_3NO_2) is the monamide of carbonic acid; it is not known in the free state, and when dissociated from its combinations it is at once resolved into carbonic acid and ammonia. The diamide of carbonic acid is uræa.

Carbamic acid has been found in the urine of healthy persons, and in increased amount when lime is added to ordinary food (Abel and Muirhead²). It is also increased in diseases which profoundly affect the function of the liver (Hahn and Nencki³).

When naturally alkaline urine which contains carbamic acid is allowed to stand for some time it gives off ammonia. The separation of carbamic acid from urine is a tedious process; it is best accomplished by Abel and Drechsel's method.⁴

PHENOL. $\text{C}_6\text{H}_5\text{OH}$.

Phenol is one of the hydroxy-compounds of the aromatic series which, according to the number of hydroxyl-groups they contain, are divided into mono-, di-, trihydric phenols; the phenol under consideration is a monohydric phenol, and is commonly called carbolic acid.

Cresol ($\text{C}_6\text{H}_4\cdot\text{CH}_3\cdot\text{OH}$), a homologue of phenol, represents most of

¹ *Bull. Soc. Chim. de Paris*, 1903.

² *Arch. d. sc. biologiques*, 1892.

³ *Arch. f. exper. Pathol.*, 1893.

⁴ *Arch. f. Physiol.*, 1891.

the phenol group that is present in human urine. It occurs chiefly in the form of *p*-cresol and it closely resembles phenol in its ordinary properties.

Both these aromatic products are formed in the course of proteid decomposition and, along with other members of the aromatic series, occur during the later stage of tryptic digestion. From the digestive tract they are almost entirely absorbed and are conjugated with sulphuric acid, forming phenylsulphuric acid, and *p*-cresol sulphuric acid, which in combination with a base, mostly potassium, are excreted by the kidneys as ether-sulphates. For clinical purposes, phenol and cresol may be regarded as one and the same thing; it is unnecessary to differentiate them, and indeed when dealing with small quantities of urine it would be impossible to do so. Of phenol and cresol together from 2 to 3 mgrms. are excreted in the urine daily; with an exclusively vegetable diet, the daily excretion is larger. All conditions which intensify the putrefactive processes in the intestines, such as ileus, peritonitis, ulceration of the bowel, and simple constipation, cause an increase in the amount of phenyl- and cresol-sulphates in the urine, as do also suppurative processes of a septic character in any of the cavities of the body—empyema for example.

In order to test for phenol and its homologue, or to estimate their amount, it is necessary that they should first be set free from their combinations. This is done by freely acidulating some of the urine with hydrochloric acid—10 c.c. to every 100 c.c. of urine—and then distilling the urine until the distillate ceases to respond to bromine-water. If an accurate estimation is desired, the distillate is exactly neutralised with caustic soda, in order to combine with and keep back benzoic acid derivatives, &c., and is then re-distilled until the phenols have come over. The last distillate, treated with excess of bromine-water, is allowed to stand for twenty-four hours. The precipitate of tri-bromo-phenol which falls is collected on a tared filter which is dried over sulphuric acid and then weighed—100 parts equal 28.39 parts of phenol.

The yellowish-white crystalline precipitate obtained with bromine-water, after distillation of the urine, may be accepted as evidence of the presence of phenol. After the addition of the first drop or two of bromine-water the precipitate may appear momentarily, and then redissolve; but as soon as the bromine is present in excess the precipitate is permanent.

PYROCATECHIN. $C_6H_4(OH)_2$.

Pyrocatechin, or catechol, ortho-dihydroxybenzene has been found in very small amount in normal urine, probably as a derivative of the phenol which is formed during the later stages of proteid decomposition in the intestines. Pyrocatechin is a crystalline substance which is soluble in water, alcohol, and ether. It is precipitated by lead acetate. It reduces Fehling's solution, but not an alkaline solution of bismuth. If a very dilute solution of ferric chloride, after the addition of a little tartaric acid, is made alkaline with ammonia and is then added to a solution of pyrocatechin, a cherry-red colour is produced which becomes green on free acidulation with acetic acid. If alkaline urine containing pyrocatechin is exposed to the air it tends to become darker in colour. Pyrocatechin may be isolated from urine by first adding hydrochloric acid and then boiling the urine until the phenol is volatilised. After cooling, the urine is extracted with ether, and the ethereal extract, on evaporation, leaves the pyrocatechin, which may be purified by being dissolved in benzene and crystallised out.

HYDROQUINONE. $C_6H_4(OH)_2$.

Hydroquinone or quinol, para-dihydroxybenzene, another of the dihydric phenols, is an isomer of pyrocatechin. It has not been found in normal urine, but it may appear after the administration of phenol by the mouth, or after its free application to the skin. Like pyrocatechin it reduces Fehling's solution, but it is not precipitated by lead acetate. It may be isolated from urine in the same way as is pyrocatechin, from which it may be separated by benzene, which dissolves pyrocatechin, but not (or only to a slight extent) hydroquinone. When acted on by ferric chloride it is oxidised to quinone, which may be recognised by its peculiar odour. The dark colour of the urine from cases of phenol poisoning, sometimes described as *carboluria*, is chiefly due to the presence of hydroquinone. On exposure of such urine to the air a decomposition-product of hydroquinone forms, by which the dark colour is produced.

Like the phenol from which they are derived, pyrocatechin and hydroquinone exist in the urine as ether-sulphates; that is, in conjunction with sulphuric acid and combined with a base, which is usually potassium.

INOSITE. $C_6H_{12}O_6 + 2H_2O$.

Inosite, or hexahydroxybenzene, may be regarded as a hexhydric phenol. It was formerly classified as a carbohydrate, and, on

account of its sweet taste, was known as "muscle sugar." It crystallises in rhombic plates, which are soluble in water, but not in alcohol, nor in ether. It is optically inactive, and it does not reduce Fehling's solution, nor is it fermentable by yeast. Inosite may be present in normal urine after excessive amounts of water have been drunk; it may also occur in diabetic and albuminuric urines.

Separation from urine.—After removal of any albumin that may be present, the urine is precipitated with lead acetate. After filtration the filtrate is concentrated on the water-bath to one-fourth its volume, and is then, whilst warm, treated with basic lead acetate as long as a precipitate forms. After standing twelve hours the precipitate is collected and is decomposed with sulphuretted hydrogen. After filtering and allowing the uric acid to crystallise out, the liquid is filtered again and is further concentrated to a small bulk, and then, whilst hot, it is treated with three or four times its volume of alcohol, which throws down a sticky precipitate that adheres to the beaker, and from it the liquid can be decanted; if the precipitate should be flocculent it must be filtered off. The liquid is allowed to stand twenty-four hours, when crystals of inosite will be deposited in groups. If no crystals form, the solution must be treated with ether until it becomes milky-looking, and again allowed to stand for twenty-four hours.

Tests.—A little of a solution of inosite is treated with some concentrated nitric acid and evaporated almost to dryness; the residue is moistened with a solution of calcium chloride, and again carefully evaporated to dryness. A rose-red colour indicates the presence of inosite, of which one milligramme can thus be detected.

If a little inosite with excess of nitric acid is evaporated to dryness, and the residue is dissolved in a little water, the addition of a small quantity of strontium acetate develops a violet coloration.

CARBOHYDRATES.

THE substances which belong to this group are: *Dextrose*, *levulose*, *lactose*, *isomaltose*, and the two pentoses, *arabinose* and *xylose*. Of these, dextrose, lactose, and isomaltose, along with a carbohydrate substance to which the name *animal gum* has been given, are found in urine, in the absence of pathological conditions. In this section it will be convenient to deal also with the pathologically associated substances, *glycuronic*, *diacetic*, and β -*oxybutyric acids*, along with *acetone*.

DEXTROSE. $C_6H_{12}O_6$.

Dextrose, glucose, or grape-sugar is soluble in water, only feebly so in alcohol, and not at all in ether. As indicated by its name, "dextrose" rotates the plane of polarised light to the right, its specific rotation $[\alpha]_D = +52.5$. It enters into combinations with alkalies, alkaline earths, and some metals, forming glucosates; when a solution of copper sulphate is added to a solution of grape-sugar, a greenish-blue precipitate is formed, which is retained in azure-blue solution by the presence of an alkali hydrate. As an aldehyde, glucose possesses strong reducing powers; consequently, if the alkaline, glucose cupric oxide is heated, a red precipitate of cuprous oxide is quickly formed, or more slowly in the cold. This property is made use of to ascertain the presence, and to determine the amount, of glucose in a solution; glucose always reduces the same quantity of cupric to cuprous oxide: 1 molecule of glucose reduces as nearly as possible 5 molecules of cupric oxide. Glucose forms an osazone with phenylhydrazin—*phenylglucosazone*, and an ester, or ethereal salt, with benzoyl chloride—*benzoyl glucose*. Glucose readily undergoes fermentation with yeast, yielding alcohol and carbon dioxide. It is not carbonised, as cane sugar is, when gently warmed with sulphuric acid. By very powerful oxidising-agents glucose is converted into saccharic acid, which by heating and subsequent reduction with sodium amalgam yields glycuronic acid.

PHYSIOLOGICAL GLYCOSURIA.

About 0.17 per cent. of glucose is present in normal human blood. A vast number of experiments have been made since Brücke¹ declared that sugar is also present in normal urine; some are in favour of, and others are against, this statement. In a complex excretion like urine, that contains several substances, each of which reacts like sugar with one or other of the tests used for its detection, convincing evidence is not easily obtained. Molisch² and Luther³ used colour tests—*alpha*-naphthol with thymol and furfuraldehyde, and obtained positive results. Wedenski⁴ and Baisch,⁵ taking advantage of the insoluble combinations which carbohydrates form with benzoyl chloride, precipitated by this reagent any glucose that might be present in urine, afterwards liberating it from the ester thus formed by treatment with sodium hydrate; they also obtained positive results. Breul⁶ and Allen⁷ precipitated glucose from urine as phenylglucosazone. Pavy⁸ precipitated with lead oxide (Brücke), and, after separation from the precipitate, obtained a substance which reduced metallic salts, reacted to phenylhydrazin and fermented with yeast. In a large number of cases Lohnstein⁹ applied the fermentation method directly to urine with positive results. Friedländer,¹⁰ Maly,¹¹ Külz,¹² and others, and more recently G. and G. S. Johnson,¹³ deny that glucose occurs in normal urine, attributing the reactions indicative of its presence to creatinin, glycuronic acid, and to carbohydrates other than glucose. It is now generally accepted that the balance of evidence is in favour of the view that normal urine may contain a minute amount of glucose. The precise quantity, as estimated by various authorities, ranges between rather wide limits: from 0.001 per cent. (Lohnstein), up to 0.05 per cent. (Pavy).

ALIMENTARY GLYCOSURIA.

Apart from the question of a trace of sugar being present in normal urine, considerable amounts may be present without the occurrence of definite pathological changes. Every healthy individual has a limit beyond which his capacity to assimilate sugar does

¹ *Wien. Akad. Sitzungsbr.*, 1858.

² *Chem. Centralbl.*, 1891.

³ *Ibid.* 1895.

⁷ *Chemistry of Urine*, 1895.

⁹ *Allg. med. Centralzeitg.*, 1900.

¹¹ *Wien. Akad. Sitzungsbr.*, 1871.

¹³ *The Lancet*, 1894.

² *Centralbl. f. d. med. Wissensch.*, 1888.

⁴ *Zeitschr. f. physiol. Chem.*, 1889.

⁶ *Arch. f. exp. Path.*, 1898.

⁸ *Physiol. of Carbohydrates*, 1894.

¹⁰ *Arch. f. Heilkunde*, 1865.

¹² *Arch. f. d. ges. Physiol.*, 1876.

not extend; when that limit is exceeded the individual excretes sugar in the urine, a condition known as "alimentary glycosuria." The limit of sugar-assimilation is not alike for all individuals, nor is it constant in the same individual even under apparently similar conditions; it is still less so under varied conditions, such as rest and work. Breul¹ gave 200 grms. of grape-sugar to a man and examined the urine he excreted during the succeeding four hours: when at rest, he excreted 2.14 grms.; when at work, only 0.09 gm. Some experiments made by v. Noorden² illustrate the effect of the individual factor in sugar-assimilation. He gave 100 grms. of grape-sugar to each of two healthy individuals, A and B; in neither of them did sugar appear in the urine. He then gave them each 150 grms., which exceeded the limit for A, who excreted 0.15 gm., but not that for B, whose urine remained free from sugar. But when each received 200 grms., A excreted only 0.26 gm., whilst B excreted 0.71 gm. The average quantity of sugar, taken in one dose, which is sufficient to produce alimentary glycosuria in a healthy man, varies with the kind of sugar. The saccharine limit is soonest overstepped by milk sugar, of which about 120 grms. are required to develop glycosuria; of cane and grape sugars over 150 to 200 grms. are needed. The alimentary glycosuria produced by a single large dose of sugar only lasts from four to five hours, when the urine is again free from sugar. It is usually stated that whatever kind of sugar is taken in excess the same kind appears in the urine, that is to say, that excess of grape sugar produces excretion of grape sugar; excess of cane sugar, excretion of cane sugar; and the same with the other varieties of sugar. This rule, however, does not apply universally; glucose may be found in the urine of healthy men to whom large amounts of cane sugar have been administered. Achard and Weyl³ point out that the test for alimentary glycosuria should always be made with grape sugar; if cane sugar is used, the result is largely influenced by the state of the digestion.

Naunyn and others speak of alimentary glycosuria arising from excess of starchy food as well as of sugar; it is difficult to believe, however, that the assimilation of farinaceous food can be accomplished so rapidly as to suddenly throw such large quantities of glucose into the blood as would be required to produce glycosuria in a *perfectly healthy* individual. It is probably correct to assume that no amount of starchy food is capable of producing alimentary glycosuria in the healthy individual; this is usually held

¹ *Arch. f. exp. Pathol.*, 1898.

² *Die Zuckerkrankheit*, 1895.

³ *Soc. méd. des Hôpitaux*, 1898.

to constitute a clear distinction between alimentary glycosuria and diabetes. J. Strauss¹ found, in fevers and in alcoholism, that alimentary glycosuria could be produced by starchy food, and expresses the opinion that alimentary glycosuria *e saccharo*, as well as *ex amylo*, is to be regarded as diabetic; it is merely a question of degree.

This leads to the consideration of those borderland cases which lie between alimentary glycosuria and pathological glycosuria. In some, sugar erratically appears in the urine, without any special dietetic provocation; in others, it may be traced to excess of carbohydrate food. Such cases are potentially diabetic and are liable actually to become so.

Various toxic agents may give rise to this partial or complete breaking down of the sugar-assimilation limit: alcohol, chloroform, nitrobenzole, amyl nitrite, carbon monoxide (?), atropin, phosphorus, arsenic, mercuric chloride, the mineral acids, and lead (chronic plumbism), are amongst them. In some—phosphorus, for example—the glycosuria is due to the effects produced on the liver, and it appears spontaneously; in others—alcohol and lead, for example—the glycosuria may only appear in response to the ingestion of much saccharine food.

Traumatic violence not unfrequently causes diabetes, and still more frequently places the patient in the borderland state. Haedke,² out of twenty-five cases of severe injury to the head, or of general physical shock, found that in fifteen the administration of 100 grms. of grape-sugar produced alimentary glycosuria. Non-traumatic disturbances of the nerve-centres may produce glycosuria, or render it easy of production. In cerebral and cerebellar tumours, apoplexy and various other lesions of the brain and cord, especially those in the neighbourhood of the fourth ventricle, glycosuria is not infrequent. It has also occurred in tabes, insular sclerosis, Graves's disease, and much more frequently in acromegaly. In general paralysis, paranoia, and delirium tremens, temporary glycosuria has been observed. In ten per cent. of hemiplegics and in five out of twenty-one melancholics, alimentary glycosuria was observed by Arndt.³ Psychical influences, such as prolonged corroding anxiety, mental shock and worry, may be followed by temporary or permanent glycosuria. Conditions due to disordered metabolism, such as gout and obesity, and also hepatic derangements, are frequently attended by glycosuria. Hofmeister⁴ described a hunger-diabetes which he observed in

¹ *Zeitschr. f. klin. Med.*, 1900.

² *Deutsche med. Wochenschr.*, 1900.

³ *Deutsche Zeitschr. f. Nervenkrankh.*, 1898.

⁴ *Arch. f. exp. Pathol.*, 1889.

animals during inanition, and G. Hoppe-Seyler¹ gives an interesting illustration of this condition in the human subject. Ten vagrants, who, on account of the state of health induced by unsettled habits, and inadequate, irregular supply of food, were admitted into hospital; in all of them sugar—in most of the cases below 1 per cent., but in one 3.5 per cent.—was present in the urine. The glycosuria speedily vanished on a mixed diet rich in carbohydrates; and having disappeared, it could not be recalled by the administration of 100 grms. of grape-sugar.

PHLORIDZIN-GLYCOSURIA.

A special form of toxic glycosuria, caused by the administration of the glucoside, phloridzin, either by the mouth or by hypodermic injection, was first observed by Mering,² who, by administering one gramme of phloridzin to a man night and morning, caused the daily excretion of nearly 100 grms. of glucose in the urine. As soon as the administration was discontinued the excretion of sugar ceased. Mering accounted for the glycosuria on the assumption that phloridzin increases the permeability of the kidneys for sugar. Loewi³ found that 2 grms. of phloridzin, given by the mouth, caused dogs to excrete 58.8 grms. of sugar; when injected subcutaneously 124.6 grms. were excreted. In phloridzin-glycosuria there is no increase of glucose in the blood. It is generally supposed that the sugar is produced in the kidneys. Minkowski⁴ found that extirpation of the kidneys in animals glycosuric with phloridzin did not materially affect the amount of glucose in the blood. Mering, and more recently Lewandowski,⁵ also found that phloridzin does not produce hyperglycæmia, but rather tends to cause hypoglycæmia. Hédon⁶ caused the hyperglycæmia of dogs with pancreatic diabetes to disappear by the injection of phloridzin. Richter⁷ states that in animals, artificially produced nephritis delays, or altogether prevents, the appearance of sugar in the urine after the administration of phloridzin. Klemperer⁸ found, in human beings with Bright's disease, that phloridzin given by the mouth did not cause glycosuria. Many of these observations are in favour of Minkowski's hypothesis that the phloridzin is split up in the kidneys into sugar and phloretin. The sugar is at once excreted and the phloretin is absorbed and combines with more sugar, which is also split off, and the process is

¹ *Münchener med. Wochenschr.*, 1900.

³ *Arch. f. exp. Pathol.*, 1901.

⁵ *Arch. f. Anat. u. Physiol.*, 1901.

⁷ *Zeitschr. f. klin. Med.*, 1900.

² *Zeitschr. f. klin. Med.*, 1888.

⁴ *Ibid.* 1893.

⁶ *Compt. Rend. Soc. Biol.*, 1897.

⁸ *Vereines f. inn. Med.*, 1896.

repeated until the phloretin is excreted. Charlier¹ obtained a ferment from the kidney of the horse, which affected the cleavage of phloridzin; but he failed to obtain one from the dog. Pavy, Brodie and Siau² consider that the theories explanatory of the mode in which phloridzin acts fail to account for the existing conditions, and attribute the effects produced by phloridzin to a specific action on the cells of the renal tubules by which they acquire the power of producing glucose in a manner comparable with the power of the mammary cells to produce lactose.

RENAL GLYCOSURIA.

In this condition the glycosuria is not due to any alteration in the carbohydrate metabolism, but to an abnormal excretion of the sugar that is normally present in the blood. According to Klemperer's view, true renal diabetes is due to a morbid activity of the renal epithelium towards sugar, which is thus passed from the blood to the urine, the amount in the blood not being excessive. As the carbohydrate metabolism is not in fault, the administration of farinaceous food, or of grape-sugar, does not increase the glycosuria. Another kind of renal glycosuria is described as being due to excessive diuresis; in this form the renal epithelium is not selective, but is simply abnormally active, and consequently gives rise to polyuria by which sugar, along with excess of other substances, is removed from the blood.

Glycosuria has been observed to follow the administration of caffein and diuretin (a double salt of theobromine and sodium salicylate), which some observers refer to the diuresis produced by these purin bodies; others, with Richter,³ consider that the glycosuria has nothing to do with the diuresis, but that it belongs to the hepatogenous group of glucosurias, in which the liver is incapable of storing up glycogen. According to this view, hyperglycaemia occurs and occasions the polyuria; not, as held by Jacoby⁴ and Klemperer, that the diuresis is the causal factor. Gobbi⁵ found that the amount of sugar in the urine, after the administration of caffein, is not proportional to the diuresis, and that the caffein renders the kidneys pervious to sugar.

¹ *Compt. Rend. Soc. Biol.*, 1901.

² *Journ. of Physiol.*, 1903.

³ *Zeitschr. f. klin. Med.*, 1898.

⁴ *Arch. f. exp. Pathol.*, 1895.

⁵ *Il Policlinico*, 1900.

ADRENAL GLYCOSURIA.

Blum¹ proved experimentally that the adrenals contain a substance which, when introduced into the circulation, is capable of producing glycosuria; the same result does not follow the reception of preparations of the adrenals into the digestive tract. The glycosuria lasts for several days, and it occurs when the animal is fed on an exclusive flesh diet, and also after prolonged absence of food. Blum regards *diabète bronzé* as a result of adrenal disease. Herter² also produced glycosuria in dogs by the injection of adrenalin into the peritoneal cavity. Paton³ considers that adrenal glycosuria is the result of diminished sugar-destruction by the tissues, and that adrenalin probably acts on the pancreas; after repeated doses have been given, tolerance ensues and no further increased excretion of sugar occurs.

PANCREATIC DIABETES.

In animals, extirpation of the pancreas is followed in six or eight hours by the appearance of glucose in the urine; as shown by Mering and Minkowski,⁴ when only a portion of the gland is removed no glycosuria may result, or if it does it is probably of a mild type. It has been found that glycosuria does not occur after removal of the duodenal end of the gland, with ligature of the pancreatic duct; nor does it follow entire removal of the gland if a piece of it is implanted and retains its vitality under the skin of the abdomen. It is therefore evident that absence of the ordinary pancreatic juice has nothing to do with the causation of the glycosuria; indeed, Sandmeyer⁵ showed, by giving portions of pancreas, along with their food, to dogs with pancreatic diabetes, that the amount of sugar in the urine is increased on account of the improved diastatic activity thus induced. In human beings diabetes has often been found to be associated with diseases of the pancreas, such as atrophy, fatty and cirrhotic changes, cysts, acute and chronic pancreatitis, malignant disease, and other morbid conditions.

This form of diabetes is supposed to be due to the absence of an internal secretion which, in addition to the pancreatic juice, is secreted by the pancreas. Nothing is known as to the nature of this internal secretion, but there are good grounds for assuming its existence, of which none is more forcible than the fact that pancreatic glycosuria is prevented by the ingrafting of a fragment of the gland under the skin. By compressing pieces of the gland,

¹ *Deutsches Arch. f. klin. Med.*, 1901.

² *New York Med. News*, 1902.

³ *Journ. of Physiol.*, 1903.

⁴ *Arch. f. exp. Pathol.*, 1889.

⁵ *Zeitschr. f. Biolog.*, 1895.

Blumenthal¹ and Herzog² obtained a juice that decomposed sugar *in vitro*, which is evidence that the pancreas possesses glycolytic properties. Umber³ regards Blumenthal's results as due to bacterial contamination. The absence of the internal secretion of the pancreas causes hyperglycæmia, hence the glycosuria. If acute nephritis is produced in a dog with pancreatic diabetes, the excretion of sugar is diminished both relatively and absolutely, and the relation of dextrose to nitrogen is considerably smaller; the lessened excretion of sugar being due to faulty performance of function by the diseased kidneys, the sugar accumulates in the blood, and consequently the hyperglycæmia is greatly increased (Ellinger and Seelig⁴).

Some of the diseases of the pancreas above enumerated as causal factors of diabetes may attack the gland without the occurrence of diabetes. At the present time there is a disposition to regard degenerative changes in certain pancreatic structures as being alone causative of pancreatic diabetes. In 1869 Langerhans described a number of cell-groups in the gland, to which the name "islands of Langerhans" has been given; these cells are supposed to be the source of the internal secretion of the pancreas which plays such an important part in the conversion of the carbohydrates in the organism; consequently, degeneration of the islands of Langerhans is regarded as the probable cause of pancreatic diabetes. (Opie,⁵ Weichselbaum⁶.)

PATHOLOGICAL GLYCOSURIA.

This term includes all forms of glycosuria which occur independently of the ingestion of large amounts of sugar and which are associated with faulty metabolism. In pathological glycosuria sugar is not only formed in the system from other carbohydrates, but it may also be derived from alimentary and systemic proteids and fats. Much difference of opinion exists on this subject; some physiological chemists refuse to accept the statement that sugar can be split off from proteids which contain no preformed carbohydrate molecule; others think that the proteid molecule itself may yield sugar. Some also accept, and some deny, the derivation of sugar from fat. By the treatment of proteid matter with potash and heat, Pavy⁷ produced a substance that resembles animal gum, and by the action of mineral acids on this product he converted it into a substance that reduces

¹ *Zeitschr. f. diätet. u. phys. Therap.*, 1898.

² Hofmeister's *Beiträge z. chem. Physiol.*, 1902.

³ *Zeitschr. f. klin. Med.*, 1900.

⁴ *Festschr.*, Braunschweig, 1901.

⁵ *Journ. of. exper. Med.*, 1901.

⁶ *Wiener klin. Wochenschr.*, 1902.

⁷ *Proc. Roy. Soc.*, 1893.

cupric oxide, responds to Moore's test, does not give the biuret reaction, yields a crystalline compound with phenylhydrazin, and forms an ester with benzoyl chloride; but it is optically inactive and it will not ferment. Pavy regards this substance as being derived from a cleavage of the proteid molecule. Seegan,¹ by warming for several hours a mixture of defibrinated blood, liver-tissue, and oil, produced an increased amount of a reducing substance which he regards as sugar. Weiss² repeated the experiment with the same result. A vast number of experiments, chiefly on animals, have since been made in order to ascertain whether sugar can be formed in the organism from proteids and fat.

By deprivation of food, and by active exercise, animals are freed from glycogen, and by means of phloridzin are made glycosuric, so that any sugar that may be formed is carried off by the urine; they are then fed on known amounts of various kinds of proteids, or of fatty food. In order to determine the relation borne by the sugar which is excreted to the proteid metabolism, the amount of nitrogen in the urine, as well as the amount of the sugar, is determined, and from the data thus obtained the sugar: nitrogen quotient, $\frac{S}{N}$, is derived. By this method, Bendix³ obtained with egg-albumin a $\frac{S}{N}$ quotient of from 1.79 to 4; with casein 3.1 to 4.3; that is, more sugar from a proteid (casein) which contains no carbohydrate radicle than from one which does. Hartogh and Schumm,⁴ administering to dogs under the same conditions fat in place of proteids, obtained an average ratio of nitrogen to sugar equal to 1:4.1; in several single experiments the sugar-value was much higher, 1:9 and even 1:13. Bouchard and Desgrez⁵ found that fat did not increase the amount of sugar in the urine, nor, as ascertained after the animal was killed, the amount of glycogen in the liver; but in other experiments the amount of glycogen in the muscles was found to be increased; they assume that glycerin, the cleavage product of fat, is converted into carbohydrate. Loewi,⁶ after experimenting with dogs under the influence of phloridzin, concludes that sugar is not derived from fat. In a case of acute diabetes, Lüthje⁷ found that various proteids were of unequal value as regards the amount of sugar excreted; the largest amount followed the ingestion of casein, and the smallest that of white of egg; these results agree with Bendix's experiments.

¹ *Zuckerbild. im Thierkörper.*, 1890.

² *Zeitschr. f. physiol. Chem.*, 1898.

³ *Verhandl. d. physiol. Gesellsch. Berlin*, 1900.

⁴ *Arch. f. exp. Pathol.*, 1900.

⁵ *Comptes Rendus*, 1900.

⁶ *Arch. f. exp. Pathol.*, 1901.

⁷ *Zeitschr. f. klin. Med.*, 1900.

LEVULOSE. $C_6H_{12}O_6$.

Levulose, or fruit sugar, is so named on account of its property of rotating the plane of polarised light to the left. In 10 per cent. solution at 20° C. it has a specific rotation $(\alpha)_D = -92^\circ.25$. Each degree of increase in temperature diminishes the specific rotation about $0^\circ.6$. Very exceptionally levulose occurs in urine alone; more commonly it is associated with dextrose. When levulose alone is present the symptoms may be of a mild type, like those of simple glycosuria, the urine not being of high specific gravity nor excessive in quantity, and not containing a large percentage of sugar. In such cases the thirst is but little above the normal and the condition is usually amenable to treatment. Cases are recorded in which the presence of levulose in urine appeared to be specially accompanied by mental depression. The administration of glucose to patients suffering from levulosuria does not necessarily increase the amount of levulose in the urine. To a woman aged 51, who passed from 1700 to 1800 c.c. of urine containing levulose and but a small amount of glucose, Rosin and Laband¹ administered 150 grms. of glucose without causing any obvious change in the urine; after the administration of 150 grms. of levulose, there was rather less lævotation than before. It has been observed that levulose is changed in the system to other carbohydrates, especially grape sugar (Hale White²). Ferrannini³ gave 100 gm. doses each of glucose and levulose to patients suffering from various liver-diseases, such as cirrhosis, syphilitic liver, cancer, and chronic enlargement, and from subsequent examination of the urine concluded that alimentary levulosuria, and not glycosuria, affords the best indication of insufficiency of the glycogen-forming function of the liver.

A sugar found in urine and described by Leo⁴ as being of the levulose type, to which the name "Laiose" has been given, was probably a pentose.

LACTOSE. $C_{12}H_{22}O_{11}$.

Lactose, or milk sugar, is dextro-rotatory and reduces Fehling's solution (more slowly than glucose), but it does not ferment with yeast. It forms a compound with phenylhydrazin, the crystals being shorter than those of glucosazone. At one period or another in the course of normal lactation, the urine of women who suckle their infants usually gives indications of the presence of sugar,

¹ *Centralbl. f. d. med. Wissensch.*, 1902.

² *Guy's Hosp. Reps.*, 1903.

³ *Centralbl. f. innere Med.*, 1902.

⁴ *Virchow's Archiv*, 1887.

especially if the breasts are gorged on account of abrupt withdrawal of the child from the nipple. The carbohydrate present in the urine is lactose, which is absorbed from the mammary glands and passes unchanged through the liver. The resulting glycosuria is not pathological, nor has it any relation to other forms of glycosuria; it is limited to the period of lactation, and is not dependent upon any derangement of metabolism.

ISOMALTOSE. $C_{12}H_{22}O_{11}$.

In many respects this sugar resembles maltose, but it differs in being unfermentable with yeast, and in the extreme solubility of its osazone, which is soluble in four parts of hot water; its isomer maltosazone requires seventy-five parts. Isomaltose is dextro-rotatory and reduces Fehling's solution. It was first described by Fischer,¹ and has been found in normal urine by Baisch² and Lemaire.³ Its occurrence in urine is not known to be of any practical import.

PENTOSE. $C_5H_{10}O_5$.

Pentose, or five-carbon sugar, was first discovered in urine by Salkowski, and Jastrowitz⁴; it has since been found occasionally to be present. The pentoses which appear in urine are *arabinose* and *xylose*; rhamnose has not been found. Pentoses are only partially assimilated when received into the system, a considerable portion being excreted unchanged in the urine. After certain fruits, such as pears and plums, have been eaten, pentoses may often be detected in the urine of healthy persons, constituting alimentary pentosuria. When pentoses are ingested, von Jaksch⁵ found that the amount excreted varied considerably, and also that the duration of the excretion ranged over wide limits. The urine of herbivorous animals whose food contains much pentose is all but free from pentoses. This led Salkowski⁶ to suppose that, although pentoses are not fermented by yeast, they may be split up by bacteria in the intestinal canal. This method of cleavage he succeeded in demonstrating by submitting pentoses to the action of an alkaline mixture of putrefying animal matter for some days, after which the pentoses were no longer recognisable, a considerable yield of alcohol having taken their place. Bendix⁷ also succeeded in decomposing pentoses by yeast combined

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1890.

² *Zeitsch. f. physiol. Chem.*, 1894.

⁴ *Centralbl. f. d. med. Wissensch.*, 1892.

⁶ *Zeitschr. f. physiol. Chem.*, 1900.

⁷ *Zeitschr. f. physik. u. diät. Therapie*, 1900.

³ *Ibid.* 1895.

⁵ *Zeitschr. f. Heilkunde*, 1899.

with bacteria and peptone or pancreas-powder, and produced alcohol, volatile fatty acids, and lactic acid with carbon dioxide.

In pentosuria the usual symptoms of diabetes are absent; that is to say, there is no thirst, no increased appetite, nor excessive polyuria. The amount of urine does not exceed 60 to 80 ounces daily, and its specific gravity is not over 1030 or 1035. The amount of pentose present is from 0.5 to 1 per cent. Small amounts of pentose not unfrequently accompany the glucose of severe diabetes, and small quantities of glucose may occur in cases of pentosuria.

The pentose that is excreted after the ingestion of fruits, constituting alimentary pentosuria, is the dextro-rotatory arabinose; whereas that which is excreted in true pentosuria has been found to be optically inactive. Neuberg¹ believes that inactive arabinose is only formed synthetically in the system; all the pentose that can be ingested as food is optically active. When urine reduces Fehling's solution and yields crystals with phenylhydrazin, but is optically inactive and will not ferment, the presence of pentose may be suspected. Only a limited number of cases of pentosuria have been recorded, and beyond perversion of metabolism of a peculiar kind little can be said as to the cause. In one case the patient was a cocaine-habitué. The observations of Bial and Blumenthal² show that pentosuria can exist without any disturbance of the metabolism of glucose. They gave 100 grms. of glucose to a pentosuric patient, the whole of which was oxidised; none appeared in the urine. Nor was the oxidising power for pentose itself diminished: of 50 grms. of arabinose administered, only 6 grms. appeared in the urine.

Animal gum, or achrooglycogen, first described by Landwehr,³ and subsequently by Baisch,⁴ Luther,⁵ and others, is a carbohydrate substance which is present in normal urine, and which resembles erythro-dextrin or glycogen. By Halliburton⁶ it is regarded as a decomposition product of mucin. Alfthan⁷ suggests that it is probably not a simple substance, but that it represents a mixture of substances which are precipitated by alcohol. It forms insoluble combinations with the alkalies, the alkaline earths, and with some metals. Its solutions have a feeble dextro-rotatory power. By prolonged boiling with dilute mineral acids, animal gum is converted into a substance that slowly reduces Fehling's solution, but it does not ferment with yeast.

¹ *Zeitschr. f. klin. Med.*, 1900.

³ *Zeitschr. f. physiol. Chem.*, 1882.

⁶ Schäfer's *Physiol.*, 1898.

² *Deutsche med. Wochenschr.*, 1901.

⁴ *Ibid.*

⁵ *Dissert.*, 1890.

⁷ *Berliner klin. Wochenschr.*, 1902.

GLYCURONIC ACID. $C_6H_{10}O_7$.

This monobasic acid is closely related to glucose, from which it may be obtained by oxidation. It belongs to the group of aldehydes, and therefore acts as a reducing-agent. It reduces ammonio-nitrate of silver in the cold, and alkaline solutions of cupric and bismuthic oxides with the aid of heat. It forms a crystalline compound with phenylhydrazin, and, like the pentoses to which it is also closely related, it does not undergo alcoholic fermentation. It conjugates with phenol, indoxyl, and skatoxyl, and in normal urine chiefly occurs as phenolglycuronic acid in combination with potassium. Free glycuronic acid is *dextro*-rotatory, but, almost without exception, its combinations are *laevo*-rotatory. Many observers have noticed that almost all normal urines have a feeble *laevo*-rotatory power, and that they also possess slight reducing properties, both of which are doubtless due to the presence of glycuronic acid combinations.

In small amount glycuronic acid occurs in normal urine. Mayer and Neuberg¹ demonstrated its presence to the extent of 4 mgrms. in 100 c.c. of urine; it was found to exist chiefly in conjugation with phenol, and, in a lesser degree, with indoxyl and skatoxyl. Mayer² believes, in many cases, that the power of the organism to oxidise glucose is so far diminished that, in part, the oxidation stops short at the formation of glycuronic acid. He found, after giving from 100 to 200 grms. of grape-sugar to patients with alimentary glycosuria, that in fourteen instances conjugated glycuronic acid along with sugar, and in six the conjugated acid without sugar, appeared in the urine. In various acute febrile diseases, and also in conditions associated with respiratory difficulty, a like increase of conjugated glycuronic acid was found. Mayer gave 10 grms. of sodium glycuronate to a rabbit, which caused both saccharic acid and a large quantity of oxalic acid to appear in the urine. He explains the occurrence of oxaluria in diabetes mellitus on the assumption that the excessive excretion of sugar is due to most of it not undergoing any oxidation at all; whilst by better sugar-assimilation part is oxidised, not to carbon dioxide and water, but only to oxalic acid. Blumenthal³ finds it difficult to understand how small amounts of glucose and of glycuronic acid can be excreted together solely because the oxidising power for these substances is diminished. He thinks that since unconjugated glycuronic acid is never present in urines, even after it has been hypodermically injected, it is probable that the formation of the substances with which it conjugates may

¹ *Zeitschr. f. physiol. Chem.*, 1900.

² *Deutsche med. Wochenschr.*, 1901.

³ *Arch. f. Physiol.* (suppl.), 1901.

precede the formation of the acid. According to this view there is primarily an increased formation of phenol and indoxyl, and subsequently of glycuronic acid which, by combination with these products of bacterial decomposition, renders them inert. By passing blood containing phenol through the liver of a dog, Embden¹ found that in addition to conjugated sulphates, phenol-glycuronic acid is formed. Excess of indoxyl is very common in diabetes, and, as shown by Strauss,² the increased excretion of glycuronic acid stands in close relation to it.

In addition to its occurrence in cases of diabetes, glycuronic acid and its derivatives appear in abnormal amount in the urine of patients who have taken large doses of chloral hydrate—as urochloral acid (trichlorethyl-glycuronic acid) and after chloroform. Morphine, salicylic acid, oil of copaiba, camphor, turpentine, and other ethereal oils, thymol, and a number of coal-tar products, impart reducing properties to urine which are dependent on glycuronic acid combinations. In addition to these non-nitrogenous conjugations of glycuronic acid, Wiedemann,³ Schmiedeberg,⁴ and others describe a nitrogen-containing glycuronic acid—uramido-glycuronic acid, which, on heating with barium hydrate, yields ammonia and carbon dioxide, along with conjugated glycuronic acid free from nitrogen.

The relation of glycuronic acid to the pentoses may be demonstrated by its oxidation with the aid of hydrogen peroxide in the presence of ferric acetate, as accomplished by Ruff,⁵ who thus produced a considerable amount of arabinose. By adding glycuronic acid to putrefying animal matter, Salkowski and Neuberg⁶ succeeded in splitting-off carbon dioxide from the acid and so converting it into l-xylose.

THE DETECTION AND ESTIMATION OF SUGAR IN URINE.

THE DETECTION OF GLUCOSE.

For clinical purposes, the methods commonly used for the detection of sugar in urine are based on (a) the reducing power of glucose, and (b) on its property of forming characteristic crystalline compounds with certain chemical reagents. Exceptionally, the fermentative and the optical properties of glucose are also appealed to. One old

¹ Hofmeister's *Beiträge z. chem. Physiol.*, 1901.

² *Deutsche med. Wochenschr.*, 1902.

³ *Arch. f. exp. Pathol.*, 1877.

⁴ *Ibid.* 1881.

⁵ *Berichte d. deutsch. chem. Gesellsch.*, 1898.

⁶ *Zeitschr. f. physiol. Chem.*, 1902.

test is occasionally used which is not founded on any of the properties above named, viz., Moore's test.

Moore's Test.—Equal volumes of the urine and of liquor potassæ (B.P.) are boiled in a test-tube. If glucose be present the liquid darkens in colour, becoming yellow or dark-brown in accordance with the amount of glucose in the urine. Any albumin in the urine must be removed by boiling (after the addition of a drop or two of acetic acid) before applying the test. The objections to this test are: that it will not detect less than about 1 per cent. of glucose, and that high-coloured urines darken although glucose is not present.

(a) **Tests Based on the Reducing Power of Glucose.**

Trommer's Test.—To a little of the urine in a test-tube a few drops of a solution of copper sulphate and then a little liquor potassæ or sodæ are added. If much sugar be present the solution becomes deep blue in colour, and on standing for several hours it deposits a red or yellow precipitate. By raising the urine to the boiling-point immediately after adding the reagents, the precipitate at once forms. In this country, Trommer's test has given place to the following method.

Fehling's Test.—In this test the copper is held in solution as a tartrate, in combination with potassium sodium tartrate, so that it is not precipitated by the caustic alkali which enters into the composition of the reagent. Fehling's solution is liable to spontaneous decomposition, and therefore is best prepared in two portions, equal parts of which are mixed together when required. Solution A consists of 34.64 grms. of pure copper sulphate dissolved in 500 c.c. of distilled water. Solution B consists of 180 grms. of Rochelle salt and 70 grms. of sodium hydrate, dissolved in about 300 c.c. of distilled water; when cold the solution is made up to 500 c.c. When the reagent is required, equal volumes of A and B are mixed and stirred together; the resulting clear solution has a deep blue colour. Fehling's solution will detect 0.02 per cent. of glucose in urine.

The solution is thus used: A test-tube is filled an inch deep with the solution, which is then boiled for a few seconds over a spirit-lamp; no colour-change is produced unless the solution has deteriorated by keeping. Assuming that no change takes place, the test-tube is withdrawn from the flame and, at the same moment, two or three drops of the suspected urine are added; if much sugar be present, a yellow or a red precipitate of cuprous oxide is quickly formed and eventually subsides to the bottom of the tube. Should no precipitate be formed, more of the urine must be added, but less

than is equal to the volume of Fehling's solution; the tube is now replaced over the flame and its contents are just raised to the boil, when it is withdrawn. Unless the percentage of sugar is very small, the liquid begins to change just as it reaches the boiling-point, turning orange-yellow or red and becoming turbid. If but a trace of sugar be present, no alteration takes place for some time, when at a certain period, the liquid ceases to be limpid and then rapidly changes from blue to milky-green. If no change of colour occurs the urine may be regarded as free from sugar. It is important to remember that the mixture of Fehling's solution and urine *should never be submitted to prolonged boiling*, as several substances which are capable of reducing copper salts with the aid of prolonged heat may one or other be present in the urine. Amongst them, apart from dextrose, are—levulose, lactose, arabinose, xylose; glycuronic, glycosuric, hippuric, uric, homogentisic, and salicylic acids; creatin, creatinin, xanthin, hydroquinone and pyrocatechin.

Heat is not necessary for the reduction of Fehling's solution by sugar; if saccharine urine is mixed with Fehling's solution, and is allowed to stand in the cold for twenty-four hours, the cupric salt is reduced. Used in this way, however, although the action of other reducing substances than sugar is guarded against, the test loses in delicacy to such an extent as to fail in the very cases where the possible presence of these adventitious substances gives rise to doubt, so that reduction in the cold is seldom resorted to. When a considerable quantity of urine is added to Fehling's solution, a precipitate of earthy-phosphates may be thrown down by the alkali of the solution; this is not to be mistaken for a reduction-product.

As already stated, the precipitate produced by heating Fehling's solution with diabetic urine may be either red or yellow, due respectively to the formation of cuprous oxide, or of cuprous hydroxide. An aqueous solution of glucose, when heated with Fehling's solution, causes a red precipitate of cuprous oxide, and many diabetic urines which contain much sugar do the same. Neumayer¹ has shown that the yellow, or orange cuprous hydroxide produced by diabetic urine, is due to the presence of creatinin. If some urine that gives a yellow precipitate with Fehling's solution is treated with mercuric chloride and sodium acetate so as to remove the creatinin, it then gives a red precipitate. Cipollina² corroborated this by boiling diabetic urine, which gave a yellow precipitate, with caustic soda for three-quarters of an hour, in order to convert the creatinin it contained into creatin, which does not give a yellow precipitate with

¹ *Deutsches Arch. f. klin. Med.*, 1900.

² *Deutsche med. Wochenschr.*, 1901.

copper salts; the portion of urine so treated gave a red precipitate. He found that, although lactic and sarcolactic acids, guanidin, glyco-cyamin, and other allied substances also cause a yellow precipitate, the substance that produces this effect in ordinary diabetic urine is creatinin, of which an extraordinarily small amount, 1 : 10,000, is sufficient to determine precipitation of the copper (by glucose) as the yellow hydroxide; and that even 1 : 20,000 produces an orange precipitate quite distinct in colour from the red cuprous oxide.

Nylander's Test.—In 100 c.c. of a 10 per cent. solution of sodium hydrate, 4 grms. of Rochelle salt and 2 grms. of bismuth subnitrate are dissolved with the aid of gentle heat; when cold the solution should be filtered. It keeps good for any length of time.

If one part of this solution is added to ten parts of urine and the mixture is boiled for a couple of minutes or more, it becomes black and deposits a black precipitate of reduced bismuth. This reagent will detect 0.05 per cent. of sugar in urine. Any albumin in the urine must be removed before the test is used.

Picric Acid Test.—If to equal volumes of urine and a saturated aqueous solution of picric acid, one-fourth the volume of liquor potassæ be added an orange-red colour results; on boiling the liquid, it deepens in colour in accordance with the amount of sugar that is present. The presence of creatinin in urine deprives this test of much of its value, as creatinin also produces a red colour.

The Phenylhydrazin Test.—In 1886 von Jaksch¹ introduced, as a clinical test, one of the methods adopted by E. Fischer² in his investigations on “Verbindungen des Phenylhydrazins mit den Zuckerarten.” Glucose, as an aldehyde, yields a hydrazone when treated with phenylhydrazin; this glucosazone is distinguished by the colour and microscopic appearance of its crystals and by its melting-point. The test has been applied in many ways; in some, the object has been to enhance its delicacy to such an extent as to cause it to reveal the presence of sugar in normal urine. For clinical purposes this would be a disadvantage; therefore, only those methods will be here described that are serviceable as regards the discovery of glucose when present in urine in abnormal amount.

Williamson's³ method is efficient and simple. In an ordinary sized, dry test-tube, phenylhydrazin hydrochloride is introduced to the depth of half an inch; to this, another half-inch layer of sodium acetate is added. The tube is then half filled with urine and is

¹ *Zeitschr. f. klin. Med.*, 1886.

² *Berichte d. deutsch. chem. Gesellsch.*, 1884.

³ *Diabetes Mellitus*, 1898.

boiled over the flame for two minutes, after which it is allowed to stand for a few hours before the deposit is examined. If sugar is present in any but a negligible (physiological) amount, typical glucosazone crystals are seen when some of the deposit from the bottom of the tube is pipetted and placed on a slide under the microscope. These crystals are canary-yellow in colour, are needle-shaped, and, unless very scanty, are usually seen in sheaves or clusters. They have a melting-point of 205° C. When no crystals are formed, the absence of sugar may be assumed.

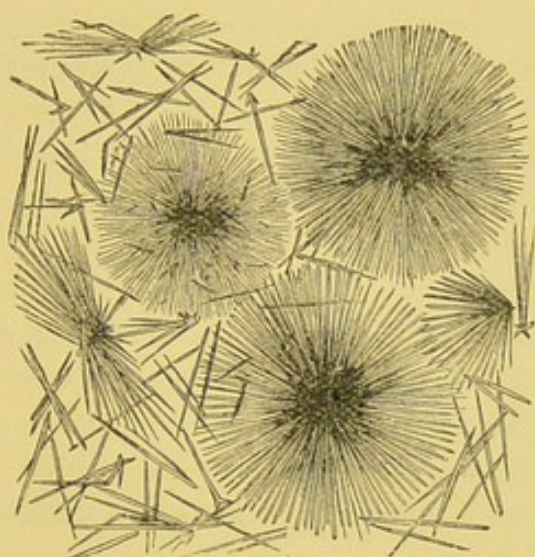


FIG. 1.—Glucosazone crystals.

For some reasons it is advantageous to use phenylhydrazin itself, instead of the hydrochloride. Neumann¹ takes 5 c.c. of urine, 2 c.c. of a saturated solution of sodium acetate in 50 per cent. acetic acid, and two drops of phenylhydrazin. The solution is evaporated down to 3 c.c., when a few drops of a solution of sodium hydrate are added in order to diminish the acidity; the solution is again heated for a moment and then allowed to cool gradually. By this method crystals are obtained with 0.01 per cent. of sugar.

Cipollina² has modified this method thus: five drops of phenylhydrazin, 0.5 c.c. of glacial acetic acid, and 4 c.c. of urine are boiled together for one minute; four or five drops of a solution of sodium hydrate (S.G. 1.16) are then added, to reduce but not to abolish the acidity of the liquid, which is again boiled for a moment and then allowed to cool. If no crystals appear in an hour, sugar in pathological amount is absent. This is a convenient and delicate test.

p-Bromo-phenylhydrazin hydrochloride gives more delicate results than the phenylhydrazin salt. If one-third of an inch deep of the

¹ *Arch. f. Physiol.*, 1899.

² *Deutsche med. Wochenschr.*, 1901.

bromo-salt with an equal bulk of sodium acetate are placed in a test-tube, which is then filled to one-third its capacity with saccharine urine, and the mixture is boiled for two minutes, crystals rather longer and paler than those yielded by phenylhydrazin are obtained. By this test distinct evidence of the presence of glucose is given by urine that yields negative or doubtful results with phenylhydrazin hydrochloride used under the same conditions; when performed as described, however, it only responds to the presence in the urine of an amount of glucose that is beyond the physiological limit.

The tests so far described, though capable of yielding excellent and trustworthy results when judiciously applied, are not free from certain fallacies. The bismuth-test is vitiated by the presence of albumin and other sulphur-containing bodies, as well as by several of the substances present in urine which are capable of acting as reducing-agents, which also affect Fehling's solution. Apart from sugar, the most important of these substances is glycuronic acid (*q.v.*); the same substance may also give an embarrassing reaction with the phenylhydrazin test. The only test that by its positive reaction affords absolute proof of the presence of sugar is the fermentation test. When used as a corroborative test, appeal is usually made to fermentation in order to decide whether an imperfect or ambiguous reaction obtained with one of the preceding tests is or is not due to sugar; this implies that, if due to sugar, it is necessarily present in extremely small amount, hence certain definite precautions are necessary to render the test decisive.

The Fermentation Test (Qualitative).—The clamp of an ordinary wooden burette-holder is arranged so as to support side by side, in a vertical position, two 18-inch lengths of barometer-tubing, both sealed at the upper end in the blowpipe flame. The tubes are filled with mercury so as to be free from air-bubbles and are then fixed in the clamp with their open ends below the surface of some mercury contained in a small trough. The urine to be tested must have been previously slightly acidulated with tartaric acid and then boiled in order to dislodge any combined carbon dioxide; and further, as pointed out by Malfatti,¹ not only is some of the carbon dioxide, which results from the fermentation, held in simple solution by the urine, but some may also become chemically combined by interchange with the monohydric sodium phosphate that is present, forming dihydric sodium phosphate and sodium bicarbonate; this possible error is avoided by acidulation, which converts any monohydric phosphate into the dihydric salt. About 5 c.c. of the urine

¹ *Centralbl. f. Krankh. d. Harn. u. Sexualorg.*, 1901.

thus prepared is mixed with a little fresh yeast that has been suspended in distilled water and, by means of a pipette, slightly curved at the delivery end and furnished at the other end with a hollow rubber ball, the mixture is delivered from beneath the level of the mercury in the trough into one of the tubes. The second tube is charged with an equal volume of water and yeast, in order to ascertain whether the yeast itself gives off gas. The apparatus is then placed in a warm room for twenty-four hours; the best temperature for fermentation is $25^{\circ}\text{C.} = \text{to } 77^{\circ}\text{F.}$ At the end of this time, any gas present in the tube charged with urine is tested by injecting a little solution of potash with the aid of the pipette; if the gas is carbon dioxide it is at once absorbed. Such a result, taken in conjunction with a negative reaction in the control-tube, proves the presence of sugar in the urine. If no gas is present in the tube containing the urine, absence of sugar having pathological significance is also proved, provided that the activity of the yeast is demonstrated. This is easily accomplished by adding some of it to a solution of glucose and placing the mixture in a third tube, under the same conditions as the other two: evolution of carbon dioxide shows that the yeast is active. By the method above described, it is easy to obtain positive evidence of the presence of sugar in urine which does not contain more than 0.1, or even 0.05 per cent.

DETECTION OF LEVULOSE.

The reactions of levulose are all but the same as those of glucose. Having the properties of a ketone it reduces copper salts more rapidly, but to the same extent as glucose: 10 c.c. of Fehling's solution represent 0.05 gm. of levulose. It ferments less rapidly than glucose with yeast, and with phenylhydrazin it forms an osazon like that of glucose. By substituting methyl-phenylhydrazin for phenylhydrazin, Newbury¹ obtained an osazone with levulose which is not yielded by glucose. This methyl-phenylosazone has a melting-point of 153°C. A solution of 0.2 gm. in a mixture of pyridine and alcohol has a dextro-rotatory power $[\alpha]_{\text{D}} = +1.40$.

*Seliwanoff's Resorcin Test*² distinguishes levulose from other sugars. If a solution of resorcin in dilute hydrochloric acid (1 : 2) is heated with levulose, the mixture rapidly becomes red and deposits a dark-coloured precipitate, soluble in alcohol, to which it imparts a red tint. Neither glucose, lactose, nor pentose gives this reaction. When levulose is present alone in urine, its property of rotating the plane of polarised light to the left, along with a positive reaction

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1902.

² *Ibid.* 1887.

with the fermentation test, are conjointly sufficient to establish the fact. When both glucose and levulose are present, the percentage of sugar ascertained by titration will not agree with the percentage given by the polariscope, because the left-rotating power of the levulose interferes with, and consequently diminishes, the right-rotating power of the glucose; hence the percentage of sugar determined by titration, including as it does both glucose and levulose, is in excess of that indicated by the polariscope. Proteids, combined glycuronic acid (the free acid is dextro-rotatory) and β -oxybutyric acid, also rotate the polarised ray to the left. Proteids can be removed by the usual methods. If, after fermentation, the urine—cleared with lead acetate—is lævo-rotatory, the presence of a substance other than levulose is indicated. On the other hand, if, after fermentation of a lævo-rotatory saccharine urine, there is loss of reducing and of optical powers, the urine contained levulose.

DETECTION OF LACTOSE.

Lactose reduces alkaline solutions of copper and bismuth salts, but more slowly than glucose. With phenylhydrazin it forms short prismatic crystals, which are usually seen agglomerated in small globular masses; the crystals of lactosazone melt at 200° C. Lactose is dextro-rotatory; it does not ferment with yeast, so that if urine which reduces Fehling's solution still retains its reducing and optical powers after being subjected to action of yeast for the usual time, and under the usual conditions, the presence of lactose, or possibly of a pentose, is indicated (*cf.* Pentoses). This negative behaviour of milk sugar, however, is only towards pure yeast (*Saccharomyces*); with many specimens of commercial yeast which contain bacteria it is decomposed into alcohol and lactic acid. Effront¹ states that the addition of 0.2 per cent. of ammonium fluoride to a fermenting fluid destroys the micro-organisms by which spurious fermentation is set up, but does not interfere with the activity of the yeast. If a solution of lactose is treated with one-half its volume of a concentrated solution of lead acetate and, after filtration, a little ammonia is added, and it is then carefully heated without boiling, a yellowish-brown colour is produced; with glucose the same test yields a red colour (Voit²).

DETECTION OF PENTOSE.

The two pentoses—xylose and arabinose—that have been found in urine reduce Fehling's and Nylander's solutions and give the

¹ *Bull. de la Soc. Chim.*, 1890.

² *Sitzungsb. d. Ges. f. Morphol. u. Physiol.*, 1889.

DETECTION OF PENTOSE.

reaction with Moore's test. They are dextro-rotatory and form osazones with a melting-point from 157° to 160° C.; but they do not undergo alcoholic fermentation. Urine which contains pentose is usually optically inactive and gives the following reactions:

The Phloroglucin Test (Ihl¹)—Salkowski² thus modifies this test. A small knife-point of phloroglucin is dissolved with gentle heat in 7 or 8 c.c. of hydrochloric acid, and, when cold, the solution is equally divided between two test-tubes. To one half, ten or twelve drops of the urine to be tested are added, and to the other is added an equal quantity of normal urine; both tubes are then put into a water-bath at the boiling-point. The tube to which the pentose-containing urine was added quickly turns a red colour, and should at

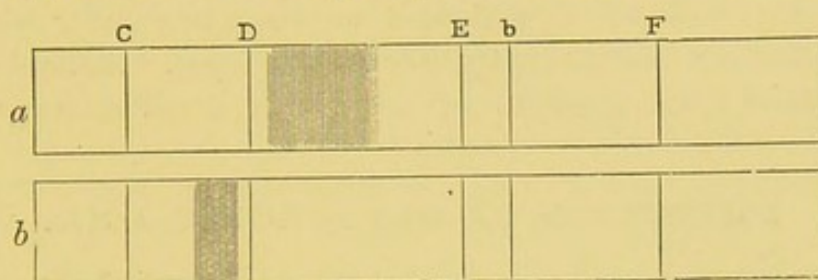


FIG. 2.

a. Pentose-phloroglucin spectrum.
b. Pentose-orcin spectrum.

once be examined with the spectroscope; it yields a band between D and E (Fig. 2, *a*). Urine which contains glucose or glycuronic acid yields the same spectroscopic reaction.

The Orcin Test (Allen and Tollens³).—To 5 c.c. of the urine a few centigrammes of orcin and 5 c.c. of hydrochloric acid are added; the mixture is heated to boiling, when a bluish-green colour appears, and, after cooling, is extracted with a little amyl alcohol which acquires a green colour and yields with the spectroscope a dark band between C and D (Fig. 2, *b*). Blumenthal⁴ and Rosin⁵ have observed a second band in the red, nearer to C. Occasionally a band appears in the green. Brat⁶ states that when the temperature is kept between 90° and 96° C. the band in the green does not develop, but that it develops if the urine is boiled; when the temperature does not exceed 96° C., and it is limited to three minutes duration, the reaction above described distinguishes pentose from glycuronic acid, which does not respond to the test when thus performed. Bial⁷ recommends the addition of a few drops of a 10 per cent. solution of ferric chloride to the orcin and hydrochloric acid in order

¹ *Chemiker-Zeitung*, 1887.

³ *Liebig's Ann. d. Chem.*, 1890.

⁵ *Med. Central-Ztg.*, 1902.

⁷ *Deutsche med. Wochenschr.*, 1902.

² *Zeitschr. f. physiol. Chem.*, 1899.

⁴ *Zeitschr. f. klin. Med.*, 1899.

⁶ *Zeitschr. f. klin. Med.*, 1902.

to help on the action. Brat states that this addition causes urines which contain no pentose, but only an increased amount of glycuronic acid, to yield the orcin reaction. Bial¹ points out that this risk is avoided by heating 5 c.c. of the reagent to the boiling-point and then withdrawing it from the flame and adding not more than one cubic centimetre of the urine.

When testing the urine for pentoses, it is not advisable to pass it through filter-paper, especially after it is acidulated, lest pentose be dissolved out of the paper.

Animal gum may be detected by its property of forming a combination with copper which is insoluble in sodium hydrate. If a solution of copper sulphate is added to urine made freely alkaline with sodium hydrate a whitish-blue precipitate falls, which, on boiling, does not become black like cupric oxide. Animal gum is distinguished from glycogen by not giving a red coloration with iodine.

DETECTION OF GLYCURONIC ACID.

When glycuronic acid is boiled for some time with a concentrated solution of potash (Moore's test for glucose), the solution becomes brown and yields the odour of burnt sugar. Urine which contains glycuronic acid compounds reduces Fehling's and Nylander's solutions. Under ordinary conditions, when the urine contains an abnormal, but not an excessively abnormal, amount of combined glycuronic acid, the reducing action does not usually occur for some short time after the suspected urine has been added to the reagent, that is, not until the two have been sufficiently boiled to liberate the acid from its combinations; by free glycuronic acid Fehling's solution is reduced as quickly as by glucose. Some conjugations of glycuronic acid, such as those of urochloral acid, are more easily split up than others, and consequently quickly reduce Fehling's solution and more readily respond to the phenylhydrazin test; indol and phenol glycuronic combinations are more resistant of cleavage. For clinical purposes, Mayer² suggests that the presence of glycuronic acid may be assumed when the urine before or, in any case, after fermentation has a lævo-rotatory power which is lessened or is changed to dextro-rotation by boiling the urine with acids; and when, after boiling with an acid, the orcin test yields a positive reaction (the reaction previously being negative) and the reducing power of the urine is increased.

By treating urine which contains conjugated glycuronic acid with phenylhydrazin hydrochloride and sodium acetate, as when testing for glucose, a crystalline combination is obtained which resembles

¹ *Deutsche med. Wochenschr.*, 1903.

² *Biochemisches Centralbl.*, 1903.

phenylglucosazone, the crystals usually being darker in colour; their melting-point is from 200° to 205° C.; after re-crystallisation it is increased to 210° C. If for phenylhydrazin, *p*-bromo-phenylhydrazin hydrochloride be substituted, along with an equal weight of sodium acetate, bright yellow crystals are produced which have the high melting-point of 236° C. when pure; before re-crystallisation they melt at 200° to 216° C. This combination was found by Neuberg¹ to possess extraordinarily high optical rotating power: a solution of it containing 0.2 grm. in 10 c.c. of a mixture of alcohol and pyridine, rotates the plane of monochromatic light (with the half-shadow apparatus) $-7^{\circ} 25'$, whilst under the same conditions other osazones and bromo-phenylosazones do not exceed from $+1^{\circ} 30'$ to $-1^{\circ} 30'$. The high melting-point, and the optical power of glycuronic acid-*p*-bromo-phenylhydrazin are characteristics which distinguish it from all other bromo-phenylhydrazin combinations.

With the orcin and phloroglucin tests free glycuronic acid yields the same reactions as the pentoses (*q.v.*); by limiting the heat to from 90° to 96° C., and its application to three minutes, as suggested by Brat,² the orcin test does not act with urine which contains glycuronic acid, as the conjugated acid does not respond, and the heat is insufficient to set it free.

Alfthan³ has devised a modification of benzoylising urine by means of which glycuronic acid may be distinguished from the pentoses: 500 c.c. of the urine are treated in the usual way with benzoyl chloride and a 10 per cent. solution of sodium hydrate, so as to form an ester, or ethereal salt, with any pentose or glycuronic acid that may be present. Subsequently the ester is saponified by treatment with sodium ethylate, by which sodium salts of pentose and of glycuronic acid are formed, the former being soluble and the latter insoluble in alcohol. If the solution yields a positive reaction with the orcin and the phloroglucin tests, the presence of pentose is indicated; if there is a precipitate which yields the same reaction, it is due to glycuronic acid.

The method adopted by Mayer and Neuberg⁴ of obtaining free glycuronic acid from its combinations in urine is as follows:—Five litres of urine are evaporated to about 300 c.c. and are then precipitated with lead acetate; the precipitate is washed and suspended in 400 c.c. of water, and, by means of sulphuretted hydrogen, is freed from lead, the gas being subsequently expelled by warmth. The solution, along with 3.5 c.c. of sulphuric acid, is heated for an hour to 100° C. in an autoclave, which may be formed out of a stoneware

¹ *Berichte d. deut. chem. Gesellsch.*, 1900. ² *Zeitschr. f. klin. Med.*, 1902.

³ *Arch. f. exp. Pathol.*, 1902.

⁴ *Zeitschr. f. physiol. Chem.*, 1900.

beer-bottle, with a stopper of the same material fitted with a rubber ring, and fastened down with wire. When cold, the solution, which has a clear yellow colour, gives the orcin-reaction very strongly, a dextro-rotation equal to 0.35 per cent. of glucose, and a bromophenylhydrazin combination with the melting-point and optical powers above described. By this process, and dealing with 50 litres of normal urine, Mayer and Neuberg demonstrated with certainty, for the first time, the presence in it of glycuronic acid.

THE ROUTINE TESTING OF URINE FOR SUGAR.

In clinical work the opportunity of examining the urine for sugar should never be neglected, although the patient may not have any symptoms indicative of glycosuria. The implied limitation formerly accepted—that when the specific gravity of the urine exceeds 1025 it should be tested for sugar—has been the occasion of many diagnostic errors. It is by no means uncommon for urine with a specific gravity at, or slightly below, 1020 to contain sugar, and occasionally it occurs with a gravity very considerably lower; v. Jaksch found sugar in urine with a specific gravity of only 1003.

In routine testing it is usually sufficient to use Fehling's solution, observing all the precautions that have been given. A negative reaction may be accepted, clinically, as proof of the absence of sugar. A distinct and readily obtained positive reaction is almost equally conclusive of the presence of sugar. Not unfrequently, however, Fehling's test gives a doubtful reaction; it may not be apparent until a minute or two after the urine has been added; or the reaction may have an ambiguous appearance. The question then becomes—is the altered appearance of the reagent due to partial reduction of the copper, and, if so, is this imperfect reduction due to glucose? In the first instance, an endeavour should be made to clear up the difficulty by having recourse to the phenylhydrazin test; if crystals are obtained they are probably due to glucose, but possibly to glycuronic acid, the fortuitous occurrence of lactose in women who are secreting milk being borne in mind. Very exceptionally, the osazone-crystals may be due to the presence of pentose in the urine. The differentiation between glucosazone crystals on the one hand, and those due to pentose and glycuronic acid on the other, lies in observing their respective melting-points; a mode of investigation, however, that is scarcely within the province of the clinical physician. In all doubtful cases, the fermentation test should be appealed to.

Quantitative estimation for sugar should always be made with

urine taken from the twenty-four hours' supply. The same rule applies to simple qualitative testing, as, in mild cases of glycosuria, sugar may be present at one or more periods of the twenty-four hours and not at others. In practice this is not always possible, and consequently the results of qualitative examination of single specimens have frequently to be accepted.

THE ESTIMATION OF GLUCOSE.

The percentage of sugar in urine may be determined in three ways: by titration with a standard copper solution, by fermentation, and by the polariscope.

TITRATION METHODS.

With *Fehling's Solution*.—A burette is charged with the urine diluted with water in accordance with the amount of sugar suspected to be present; the usual dilution consists of one volume of urine to nine of water. A porcelain basin containing 10 c.c. of Fehling's solution, diluted with 20 or 30 c.c. of water, is placed on a tripod over a Bunsen flame. Whilst the Fehling's solution is boiling the urine is gradually added from the burette; as the blue colour pales the urine must be added more slowly, the basin being tilted a little after each addition so as to enable a more exact estimation of the colour to be made by viewing some of the upper stratum of the liquid when free from the cuprous oxide. The end-point is arrived at when the liquid is colourless, which indicates that all the copper has been reduced. The quantity of urine which has been necessary to accomplish this is then read off on the burette; it is equal to 0.05 grm. of glucose. The urine having been diluted, the number of cubic centimetres used must be divided accordingly. For example, if 17 c.c. were used, and the dilution was one in ten, the actual amount of urine would be 1.7 c.c. The percentage is ascertained by multiplying 0.05 by 100 and dividing by the quantity of urine required to effect the reduction; thus $\frac{0.05 \times 100}{1.7} = 2.9$ per cent.

This method is objectionable on account of the slow subsidence of the finely divided red cuprous oxide, which makes it difficult to determine the exact moment when the blue tint disappears. To obviate the difficulty Vasey¹ recommends the addition of a couple of teaspoonfuls of barium sulphate to the diluted Fehling's solution, by which the cuprous oxide is carried down, and consequently the colour of the supernatant liquid can readily be determined.

¹ *The Lancet*, 1903.

With *Pavy's Solution*.—By this method the cuprous oxide is held in solution by ammonia, instead of being precipitated, and as its solution is colourless, the moment of disappearance of the original blue colour can be more exactly determined than with Fehling's solution.

Pavy's Solution is thus prepared :—4.158 grms. of pure copper sulphate are dissolved in 100 c.c. of warm water and then allowed to cool ; 20.4 grms. of Rochelle salt and the same quantity of caustic potash are dissolved in another 100 c.c. of water, and are added to the solution of copper sulphate. To the mixed solution 300 c.c. of strong liquor ammoniæ (s.g. 0.880) are added and the whole is made up to 1000 c.c. This solution represents one-tenth the amount of glucose that is represented by Fehling's solution ; that is to say, that 10 c.c. of Pavy's solution are reduced by 0.005 grm. of glucose. The solution may be kept for an indefinite time without deteriorating.

When performing titration with Pavy's solution, it is necessary to use a flask instead of an open basin, in order to limit the access of air to the solution and to restrict the evolution of ammonia. The flask is furnished with a rubber stopper perforated by two holes : through one the delivery-tube of the burette passes, and through the other a length of glass tube—preferably furnished with an exit valve—by which the ammonia vapour is carried off to a flask containing cold water. The burette is filled with the urine which has been diluted ten, twenty, or thirty times, in accordance with the amount of sugar that it is supposed to contain. Into the flask 10 c.c. of the ammonio-cupric solution, diluted with twice its volume of water, is poured and heat is applied ; as soon as the solution boils the urine is run into the flask until the blue colour disappears, the liquid then presenting the appearance of pure water. The end-reaction occurs at the moment the last trace of colour disappears. The percentage of sugar is calculated as described when Fehling's solution is used, the difference in oxidising value being borne in mind ; Pavy's solution represents only one-tenth the value of Fehling's solution.

The disadvantages of Pavy's method are : the evolution of ammonia-fumes and the rapid re-oxidation of the ammonio-cuprous solution by contact with air, which causes the solution to turn blue again so quickly as to render the results uncertain unless the most rigid precautions are taken. Both these disadvantages are avoided by substituting Gerrard's process : no fumes are given off, and re-oxidation with return of the blue colour is so much slower as to interpose no difficulty in the determination of the end-reaction. Gerrard's process is by far the most convenient method of titrating diabetic urine.

With *Gerrard's process*¹ as modified by Allen.²—When potassium cyanide is added to boiling Fehling's solution a double cyanide of potassium and copper is formed; the solution is then colourless, or nearly so, and when boiled with glucose it gives no precipitate of cuprous oxide. If more Fehling's solution is added than is sufficient to react with the cyanide, the extra portion is capable of being reduced by glucose, but the oxide which is formed is held in solution as is the case with Pavy's method. The absence of fumes, and the slow re-oxidation of the decolorised solution when exposed to the air, enable the operation to be performed in an open basin.

In a porcelain basin 10 c.c. of Fehling's solution, diluted with 40 c.c. of water, are heated to boiling, when a 5 per cent. solution of potassium cyanide is gradually added with stirring, until the blue colour is nearly destroyed. Then 10 c.c. more of Fehling's solution are added, and whilst the liquid is still boiling, the urine in a dilution of one volume made up to ten with water is rapidly run in from the burette, with constant stirring. The end-reaction is indicated by the disappearance of the blue colour. As the second 10 c.c. of Fehling's solution are alone reduced, they represent the total oxidising value, *i.e.*, 0.05 grm. of glucose.

It is absolutely necessary to keep the solution boiling whilst the cyanide is added and to avoid excess; the blue colour should barely disappear.

As Allen points out, the potassic cupric cyanide remains unchanged for some weeks, so that a supply may be kept ready for use: 100 c.c. of Fehling's solution are diluted with about 300 c.c. of water and decolorised as above described; the solution, made up exactly to 500 c.c., is preserved in a well-stoppered bottle. To 50 c.c. of this solution 10 c.c. of Fehling's solution are added, and the titration is performed as above described.

FERMENTATION METHODS.

The well-known fact that, by fermentation with yeast, glucose is decomposed into alcohol and carbon dioxide, has been utilised in three different ways for the purpose of quantitative estimation of glucose in urine: (1) By calculations deduced from the difference in the specific gravity of the urine before and after fermentation; (2) by measurement of the volume of carbon dioxide that is given off; and (3) by determination of the amount of alcohol produced.

(1) In 1861, W. Roberts³ published his method of determining the amount of sugar in urine by taking the specific gravity before

¹ *Pharm. Journ.*, 1892.

² *Loc. cit.*

³ *Edin. Med. Journ.*, 1861.

and after fermentation. He found that every degree of gravity that was lost very nearly represented one grain of sugar per ounce of urine. The method is exceedingly simple and easy of execution, but the result cannot be ascertained until the following day. A twelve-ounce bottle is half filled with the urine to be examined along with two or three small pieces—the size of a cob-nut—of fresh yeast; the bottle is lightly closed with a cork, nicked in such a way as to allow of the escape of gas. Another six ounces of the same urine, but without yeast, is put into a second bottle, which is corked; both bottles are placed side by side in a warm room and are left for twenty-four hours. At the expiration of this time, the fermented urine is filtered and its specific gravity is taken, the specific gravity of the unfermented urine being taken at the same time. As before stated, the difference in the number of degrees of gravity between the fermented and the unfermented urine represents the number of grains of sugar in the ounce. For example, if the specific gravity of the unfermented urine was 1035, and that of the fermented urine is 1005, the urine would contain thirty grains of sugar to the ounce. The amount per cent. is ascertained by multiplying the number of grains per ounce by the factor 0.23. Some subsequent investigators give higher and lower factors, but accept Roberts's factor for urines containing over 0.5 per cent. of sugar (Guttman¹). Before taking the gravity, it is advisable to test the fermented urine with Fehling's solution in order to make sure that all the sugar has been decomposed; if it has, the reaction with Fehling's solution is negative. The temperature, both of the fermented and the unfermented urine, should be the same when the gravity is taken, which is most accurately ascertained by means of a Westphal's specific gravity balance.

(2) Many instruments have been devised for the purpose of measuring the amount of carbon dioxide yielded by the fermentation of saccharine urine and thereby determining the percentage of sugar it contains. Of these Lohnstein's² fermentation-saccharometer is the most recent and the best (Fig. 3). It consists of a U-tube, on one limb of which is etched an empirical scale giving the percentage of sugar and thus avoiding the necessity for calculation. In order to facilitate the evolution of the carbon dioxide an air-space is left in the fermentation limb, in the top of which is a glass stopper with an air-channel, so arranged that, by turning the stopper, the communication between the tube and the surrounding air can be made or broken at will. The urine, mixed with a small quantity of yeast, is poured down the open limb (the stopper of the other limb being

¹ *Deutsche med. Wochenschr.*, 1890.

² *Berlin. klin. Wochenschr.*, 1898.

open) until the zero-mark is reached ; the stopper is then closed and 4 or 5 grms. of mercury are poured down the open limb, which by collecting in the narrow part of the tube that connects the two limbs seals off the one from the other.

The apparatus is placed in a vessel of water heated to 35° or 40° C. ; this hastens the fermentation so that it is complete in six or seven hours. The reading is taken without allowing the apparatus to cool. If the urine contains more than 1 per cent. of sugar it is correspondingly diluted, the reading of the saccharometer being multiplied accordingly.

(3) The amount of alcohol produced by the fermentation of a given quantity of saccharine urine has been estimated by means of the vaporimeter, and the result calculated as sugar ; this may be accomplished with some degree of accuracy, but the method is quite unsuited for clinical use.

POLARISATION METHOD.

The property possessed by sugars of rotating the plane of polarised light proportionally to the amount contained in the solution under examination, is utilised for the purpose of determining the percentage of sugar that is present. The rotation produced by one gramme of a substance dissolved in one cubic centimetre of liquid, and examined in a layer one decimetre in thickness, is called the "specific rotating power" of that substance, and is expressed by the formula $[\alpha]_D$. Some substances, such as glucose, rotate the plane of polarised light to the right, which is indicated by the + sign ; others, as levulose, rotate it to the left, indicated by the - sign. The instrument used for the purpose of estimation by this method is called the polarimeter, and that which yields the most accurate results is known as the "half-shadow" polarimeter. The polarimeter is an instrument so constructed that a glass tube of a definite length, 1, 2, or 3 decimetres, filled with the liquid to be examined, can be placed horizontally in the track of the polarised ray, between the polarising and the analysing sections. Before the tube which contains the urine is placed in position, the vernier attached to the eye-piece is made to

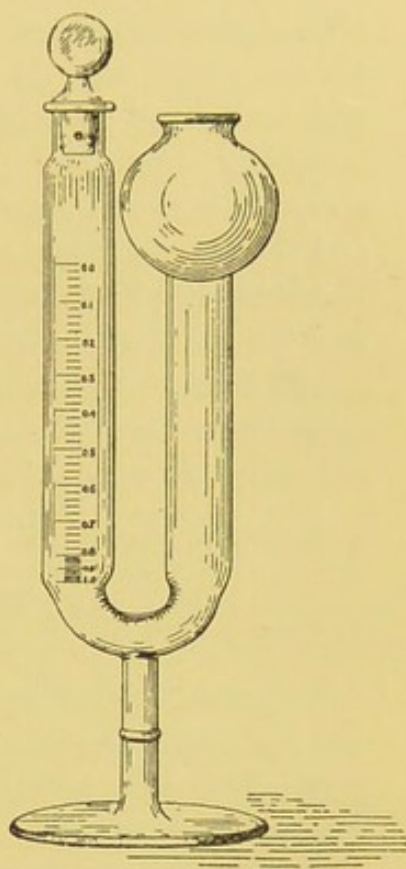


FIG. 3.

Lohnstein's Saccharometer.

coincide with the zero point; then, on focusing the eye-piece so that the vertical line by which the field is divided into two halves is distinctly seen, the two halves should appear equally illuminated; if they are not, the position of the prisms must be altered by means of an adjustment-screw, provided for the purpose, until they are equal. The tube filled with the saccharine urine is now placed in position; on re-focusing and looking through the eye-piece the field will appear unequally illuminated, one half being brighter than the other. The eye-piece is then rotated until the whole field is equally illuminated, and the altered position of the prisms is read off with the aid of the vernier. The difference in degrees between the new position and the zero point indicates the rotatory power of the sugar present in the urine. For example, the rotation amounts to 2° ; the specific rotatory power of glucose is 52.5° , that is to say, a rotation of 52.5° indicates 100 grms. of glucose in 100 c.c. of water. Therefore, with a tube one decimetre long, 1° indicates $\frac{100}{52.5}$ grms. of glucose in

100 c.c.; then $\frac{100 + 2}{52.5} = 3.8$, the percentage of sugar in the urine.

The same kind of light should always be used. Some polarimeters are constructed so that they can be used with ordinary lamplight; it is preferable, however, to use monochromatic light. For producing monochromatic light a Bunsen flame is allowed to play on some sodium salt, and the luminous flame thus produced is placed directly opposite the far end of the polariscope, which is preferably used in a dark room. The urine should be perfectly clear and not dark-coloured. When it is dark or turbid, it should be cleared by lead acetate and filtration. When a solution of lead acetate is used allowance must be made for the dilution of the urine, certain proportions of urine and of the solution being used. When filling the tube with the urine care must be taken to have it absolutely full before the glass disc, with which it is closed, is slid on to the end, and the inclusion of air-bubbles must be avoided. The amount of glucose in urine may be under-estimated by the polarimeter on account of the presence of β -oxybutyric acid, conjugated glycuronic acid, proteids, or of levulose, all of which rotate to the left, and thus tend to neutralise the dextro-rotation of glucose. A trace of proteid substance does not interfere; but when more is present it should be removed by slight acidulation with acetic acid and boiling. Oxybutyric and glycuronic acids reveal themselves, after fermentation of the urine, by rotating the polarised ray to the left. The presence of levulose along with glucose is detected by the combination of polarimetry and titration: if the latter indicates considerably more

sugar than the former, levulose is probably the cause of the discordant results.

A special form of polarimeter is made, called a saccharimeter, in which the percentage of sugar can be directly read off from the scale, the graduations being made to represent percentages of sugar in place of degrees of arc.

β -OXYBUTYRIC ACID, DIACETIC ACID, AND ACETONE.

For clinical reasons it will be convenient to consider these substances together, as their occurrence in urine is closely associated. Of the three, acetone alone may be present in normal urine, and under certain pathological conditions it may be present in considerable amount, when it may be accompanied by diacetic and β -oxybutyric acids. These substances were formerly supposed to be derived from the decomposition of proteids in the intestinal canal; or, according to v. Noorden, of the tissue proteids. The tendency at the present time is to regard them as products which are formed during the splitting up of the fat in the tissues generally, according to some authorities especially in the muscles and large glands, such as the liver. Others hold that fatty substances which undergo cleavage in the digestive tract furnish acetone if not β -oxybutyric acid. Blumenthal and Neuberg¹ return to the older view, and state that acetone may be derived from proteids as well as from carbohydrates and fat. Geelmuyden² found that in healthy human beings large amounts of fatty food cause a considerable amount of acetone to appear in the urine. Diabetics who eat large amounts of fat, especially butter, excrete much acetone, which Hagenberg³ attributes to the adventitious presence, in the edible fat, of some of the lower fatty acids rather than to pure fat. Schwarz⁴ states that, in well-nourished, healthy people, fatty food only occasions a minute increase in the amount of acetone excreted; whilst in severe diabetes acetone is increased both by fatty food and by tissue-fat, the latter being probably the source of acetone in starvation and malignant disease. Waldvogel⁵ found, after complete absence of food for a time, that the administration of 100 grms. of proteids did not cause any increase in the amount of acetone. He observes that the urea-nitrogen and acetone do not run on parallel lines; the former is greatly diminished by complete abstinence, whilst

¹ *Deutsche med. Wochenschr.*, 1901.

² *Skand. Arch. f. Physiol.*, 1900.

³ *Centralbl. f. Stoffwechsel. u. Verdauungskr.*, 1899.

⁴ *Deutsch. Arch. f. klin. Med.*, 1903.

⁵ *Zeitschr. f. klin. Med.*, 1899.

the latter is increased. β -oxybutyric and diacetic acids represent the lower stages of the decomposition of fat, and are excreted as such, either in consequence of diminished oxidation power or of excessive metabolism, so that the gravity of a case of diabetes should be estimated by the oxidising power of the tissues as regards the fat that is consumed as well as the carbohydrates. Geelmuyden regards the increased excretion of acetone during starvation as being due to the want of carbohydrate food. He took about 20 grms. of diacetic acid and found, when he lived on a mixed diet, that only 0.73 per cent. was excreted as acetone in the urine, whereas if he deprived himself of carbohydrates 6.93 per cent. was excreted; so that the capacity of the organism to decompose diacetic acid is lowered by the absence of carbohydrates from the diet. These experiments afford an explanation of the clinical experience—that it is dangerous to put a diabetic patient abruptly on a rigid dietary—*i.e.*, suddenly to cut off all carbohydrates lest coma supervene. The same conditions which diminish the power of the tissues to deal with diacetic acid would act similarly with respect to β oxybutyric acid.

Diabetic coma has been attributed—in addition to other agencies which produce no characteristic changes in the urine—to the action of acetone, of diacetic acid, and of β -oxybutyric acid. At the present time two views are held: (*a*) that the coma is due to acidosis or excess of acid—chiefly β -oxybutyric acid—in the blood; that is to say, that the condition is one of acid poisoning; (*b*) that it is due to the action of some unknown toxin.

(*a*) As long ago as 1884 Frerichs,¹ and subsequently Dreschfeld,² showed that neither acetone nor diacetic acid could intrinsically produce coma. About this time, Stadelmann³ attributed the coma to β oxybutyric acid, which he discovered in urine; this view has been supported by Minkowski,⁴ Külz,⁵ and others, and more recently by Waldvogel⁶ and Magnus-Levy.⁷ Acidosis assumes that a large excess of acid is formed in the tissues, which requires more alkali for its neutralisation than the system can supply from its ordinary resources; the result is that most of the excess of acid combines with the ammonia, derived from proteid metabolism, which otherwise would have been converted into urea. Notwithstanding this the alkalinity of the blood and the tissues becomes reduced, a condition which, according to the acidosis theory, gives rise to the coma.

¹ *Ueber d. Diabetes*, 1884.

³ *Arch. f. exp. Pathol.*, 1883.

⁵ *Zeitschr. f. Biolog.*, 1884.

⁷ *Arch. f. exp. Pathol.*, 1899 and 1901.

² *Brit. Med. Journ.*, 1886.

⁴ *Ibid.* 1884.

⁶ *Zeitschr. f. klin. Med.*, 1899.

Stadelmann¹ pointed out that the amount of ammonia in the urine is determined by the amount of oxybutyric acid that has been formed. Magnus-Levy considers (assuming no alkaline treatment has been adopted) that the ammonia closely expresses the excess of acid excretion; the ordinary amount of ammonia daily present in combination with inorganic acids does not exceed 1, or, with excessive proteid diet, 2 grms.; all above this is in combination with organic acids. In fatal cases of diabetic coma, only a moderate quantity of oxybutyric acid will be found in the urine; the surplus is locked up in the tissues. In threatened death from coma, followed by recovery, as much as 100 to 200 grms. of this retained acid may be eliminated in the urine during the progress of recovery, the elimination being furthered by the administration of large doses—sometimes exceeding three ounces—of sodium bicarbonate.

(b) Klemperer² and v. Noorden³ are the chief supporters of the toxin theory; they consider that the coma is not due to diminished alkalinity of the blood, but that the lowered alkalinity is a result of the comatose condition; in other words, they regard the coma, with its accompanying oxybutyric acid, diacetic acid, and acetone as collectively due to the action of some unknown toxin. On purely theoretical grounds, Sternberg⁴ supposes that β -amidobutyric acid, if not the toxin, is its potential representative, and is the antecedent of β -oxybutyric acid. Grube⁵ considers that β -amidobutyric acid can produce a condition like that seen in diabetic coma, but leaves it an open question whether or not β -amidobutyric acid is generally formed in the diabetic organism.

β -OXYBUTYRIC ACID. $C_4H_8O_3$.

β -oxybutyric acid is a monobasic acid which, along with its salts, rotates the plane of polarised light to the left. In the organism it is probably derived from the decomposition of fat, and is supposed to be formed in the muscles and large glands, and not in the intestinal canal; it is not present in normal urine. When submitted to the action of strong oxidising agents it yields acetone. Waldvogel⁶ found that when β -oxybutyric acid, obtained from the urine of a diabetic patient, was given by the mouth to rabbits, their urine on distillation yielded acetone. Magnus-Levy regards oxybutyric acid as a source of acetone. In severe cases of diabetes, apart from coma, as much as 20 to 30 grms. may be excreted in the twenty-four

¹ *Arch. f. exp. Pathol.*, 1886.

³ *Die Zuckerkrankheit*, 1895.

⁵ *Arch. f. exp. Pathol.*, 1901.

² *Berlin. klin. Wochenschr.*, 1899.

⁴ *Zeitschr. f. klin. Med.*, 1899.

⁶ *Centralbl. f. innere Med.*, 1898.

hours; by the administration of large doses of sodium bicarbonate the amount may be increased to 60 grms. The proportion present in diabetic urine rarely exceeds 0.5 to 1 per cent. (Magnus-Levy¹). β -oxybutyric acid has also been found in the urine in cases of scarlet fever, measles, uræmia, gastro-enteritis, and in some forms of malignant disease.

Detection.— β -oxybutyric acid is not easy to detect in urine, and, as it is always accompanied by diacetic acid, which is readily detected, the first step is to add to the urine a few drops of a solution of ferric chloride: if by this test the presence of diacetic acid is indicated, search for oxybutyric acid may then be made; in the absence of the diacetic acid reaction it is useless to proceed further.

Bergell² has simplified the method of separating oxybutyric acid from urine. From 100 to 300 c.c. of the urine, slightly alkalisied with sodium carbonate, are evaporated to a syrup, which, on cooling, is rubbed with some syrupy phosphoric acid, the mixture being kept cool, and then with 20 to 30 grms. of ignited and finely-powdered copper sulphate, and 20 to 25 grms. of very fine sand. The dry substance, in quantitative amount, is put into a Soxhlet apparatus and is completely extracted with ether that has been well dried with copper sulphate; this takes about an hour. After filtration, the solid matter on the filter is washed with dry ether which is then added to the filtrate, and the whole is evaporated down, the residue being dissolved in 20 c.c. of water; the aqueous solution is decolorised with a very little animal charcoal and its lævo-rotatory power determined. According to Magnus-Levy,³ the specific lævo-rotation is -24.12° . Külz⁴ gives it as -23.4° , and Minkowski⁵ as -20.6° .

Külz⁶ showed that when β -oxybutyric acid is heated with dilute sulphuric acid it is converted into α -crotonic acid; on this decomposition Darmstaedter⁷ bases a method of quantitatively estimating β -oxybutyric acid: 100 c.c. of urine, made feebly alkaline with sodium carbonate are evaporated on the water-bath nearly to dryness. The residue is transferred to a large flask along with 150 to 200 c.c. of 50 per cent. sulphuric acid; through a double-bored stopper, the flask is furnished with a condenser and a drop-funnel. Heat is applied at first gently, to avoid excessive frothing, and then more powerfully, water being dropped in, by means of the funnel, as distillation goes on, until 300 to 350 c.c. pass over. The distillate is extracted two or three times with ether, which is then evaporated,

¹ *Loc. cit.*

³ *Arch. f. exp. Pathol.*, 1901.

⁵ *Arch. f. exp. Pathol.*, 1884.

⁷ *Zeitschr. f. physiol. Chem.*, 1903.

² *Zeitschr. f. physiol. Chem.*, 1901.

⁴ *Zeitschr. f. Biol.*, 1884.

⁶ *Loc. cit.*

and the residue is heated to 160° C. on a sand-bath to drive off any fatty acids. The crotonic acid thus produced is dissolved in 50 c.c. of water and, after filtration, is titrated with decinormal sodium hydrate, with phenolphthalein as the indicator: 100 c.c. of the sodium hydrate solution is equivalent to 0.86 gm. of crotonic acid, which, when multiplied by 1.21, gives the amount of β -oxybutyric acid.

Pavy,¹ after obtaining a positive reaction with ferric chloride, compares the result obtained with the polariscope with that given by titration. The lævo-rotatory power of oxybutyric acid acts antagonistically to the dextro-rotatory power of the glucose; hence, if oxybutyric acid is present, the results respectively given by the optical and the reduction methods will differ in proportion to the amount of oxybutyric acid contained by the urine. This method assumes the absence of other lævo-rotatory substances, and, as pointed out by Magnus-Levy,² the amount that it gives is greatly in excess of the amount of oxybutyric acid that can be extracted from acidulated urine by ether. Another source of error arises from the use of lead acetate to decolorise the urine for polariscopic examination; an excess increases its lævo-rotatory activity. Külz³ adopts the method of removing the dextrose by fermentation, and then directly estimating the amount of oxybutyric acid in the fermented urine with the polariscope. It is doubtful how far the optical examination of urine, in order to determine the amount of oxybutyric acid, is capable of yielding accurate results; the more trustworthy plan is to isolate the acid before estimation.

Stadelmann⁴ deduces the amount of β -oxybutyric acid from the excess of bases present in the urine. Under normal conditions the sum of the acid-equivalent of urine is slightly in excess of that of the bases; but the amount of acids excreted cannot exceed that of the bases, inasmuch as free acid does not exist in urine. The acids comprise hydrochloric, sulphuric, phosphoric, and uric; the bases sodium, potassium, calcium, magnesium, and ammonia. When organic acids occur in urine, there is a corresponding excess of bases with which the organic acids are combined; this excess constitutes a scale by which the amount of organic acid may be estimated. Stadelmann found that an excess equal to 1 gm. of soda corresponds to 4.52 grms. of β -oxybutyric acid.

¹ *The Lancet*, 1902.

² *Arch. f. exp. Pathol.*, 1901.

³ *Zeitschr. f. Biol.*, 1887.

⁴ *Ueber den Einfluss der Alkalien auf den menschl. Stoffwechsel*, 1890.

DIACETIC ACID. $C_4H_6O_3$.

Diacetic or aceto-acetic acid is not present in normal urine, but it occurs under the same abnormal conditions and diseases in which β -oxybutyric acid is found. It appears chiefly in combination with ammonia, and is partly concerned in the production of the acidosis of diabetic coma. As previously stated, diacetic acid always accompanies oxybutyric acid in the urine, but it may be present apart from oxybutyric acid. At a temperature of 100° C. diacetic acid decomposes into acetone and carbon dioxide; its salts also, even in the dry state, undergo decomposition (Ceresole¹). In urine, at the ordinary temperature, it rapidly disappears, sometimes in a few hours, usually within twenty-four hours, so that the urine to be examined for it must be quite recent. Diacetic acid has been found to persist in the urine of a diabetic patient when, by strict diet, the sugar has been absent for some time (Weintraud²). Diacetic acid is usually present in the urine from those cases of chronic disease in which acetone is found; but unless the acetone odour is perceived, diacetic acid is not usually sought for and hence escapes detection. It occurs during inanition, and disappears when the starving person is well fed. It is plentiful in some infectious diseases, especially of a septic nature.

Detection in urine.—When a solution of ferric chloride is added to urine which contains diacetic acid a deep-red tint, like that of claret and water, is produced; by boiling, the colour is diminished or possibly destroyed. On the addition of the reagent, a precipitate of ferric phosphate is usually produced, which may be removed by filtration or, more readily, by dissolving it in excess of the reagent. The urine of patients who are taking salicylates, salol, phenacetin, antipyrin, or other synthetic drugs derived from coal-tar, also becomes coloured on the addition of ferric chloride, but the tint is different, being more blue-red or purple, and it deepens in place of diminishing when the urine is heated. If a patient is taking a drug of this kind, it is useless to test the urine with ferric chloride for diacetic acid. When a doubtful reaction for diacetic acid is obtained, the urine should be tested for acetone; a positive reaction indicates that the coloration produced by ferric chloride was probably due to diacetic acid.

If, after acidulation with sulphuric acid, about 50 c.c. of urine that contains diacetic acid are extracted with ether, and after separation the ether is shaken with dilute ferric chloride solution,

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1882.

² *Arch. f. exp. Path.*, 1894.

the reagent is tinted violet or Burgundy-red ; the colour disappears or lessens on warming ; if due to any of the above-named drugs it does not.

The *quantitative estimation* of diacetic acid in urine is deduced from the amount of acetone it yields, into which the diacetic acid is converted in the process of distillation.

ACETONE. $\text{CH}_3\cdot\text{CO}\cdot\text{CH}_3$.

Acetone, or dimethyl ketone, is a volatile, odorous liquid which is present in traces in normal urine, not exceeding 0.01 grm. in the twenty-four hours (v. Jaksch¹). Under various abnormal conditions the amount may be increased, the increase being probably due to excessive formation and not to diminished oxidation. If but a small excess is present in the organism, it may be perceived by the odour of acetone that is given off by the patient's breath (like the breath of a person who has been inhaling chloroform) before it can be detected in the urine. Geelmuyden² states that the oxidising power of the tissues over acetone is insufficient to deal with more than about 2 m.g. of acetone to the 100 c.c. of blood, which is approximately the normal limit ; any excess finds its way into the urine. Under abnormal conditions the amount of acetone may be very great ; as much as 5 grms. have been detected in diabetic urine in the twenty-four hours, a considerable portion being present as diacetic acid.

Whilst admitting, as contended by Blumenthal and Neuberg,³ the chemical possibility of obtaining acetone from proteids, there are valid reasons for believing that it is chiefly derived from the decomposition of fat. Some suppose that the butyric acid, which is formed in the digestive canal by bacterial action on fats, becomes converted into the optically active β -oxybutyric acid, an antecedent of acetone. Schwarz⁴ found, in diabetics, a considerable increase in acetone excretion after the ingestion of butter and also of sodium butyrate ; he calculated that, in the diabetic organism, 45 grms. of acetone were produced out of 2.5 kilo. of butter. Geelmuyden⁵ found a like increase. Hagenberg⁶ believes that pure fat does not increase the output of acetone, but that fat which contains some of the lower fatty acids does. Geelmuyden found that, in dogs with phloridzin-diabetes, the subcutaneous injection of butyric acid did

¹ *Zeitschr. f. phys. Chem.*, 1882.

² *Ibid.* 1896.

³ *Deutsche med. Wochenschr.*, 1901.

⁴ *Centralbl. f. Stoffwechsel-u. Verdauungskr.*, 1900.

⁵ *Skand. Arch. f. Physiol.*, 1900.

⁶ *Centralbl. f. Stoffwechsel-u. Verdauungskr.*, 1900.

not cause any increase in the amount of acetone. Blumenthal,¹ whilst admitting that the fat which is received into the stomach may be a source of acetone, denies that the tissue-fat can be converted into acetone. On the other hand, Schuman-Leclercq,² after a protracted series of experiments on himself with various foods and during inanition, comes to the conclusion that the decomposition of fat is the most important and perhaps the sole cause of acetone excretion, and that it is immaterial whether the fat is derived from the tissues or from food. In well-nourished, healthy people, Schwarz³ found that fatty food occasions only a minute increase, if any, in acetone-excretion; whilst in severe diabetes a distinct increase is produced both by fatty food and by the body fat. He further states that in the formation of acetone-bodies the intestinal canal is not of extreme importance.

Among the conditions in which acetone has been found in excess are: inanition, malignant disease, febrile conditions, uræmia, chronic plumbism, asthma, digestive derangements, internal hæmorrhages, narcosis produced by narcotics and anæsthetics, in lying-in women, especially when the fœtus is dead, in some psychoses, and particularly in diabetes.

The amount of acetone and diacetic acid that is excreted in diabetic urine bears no relation to the percentage of sugar that may be present, and although the presence of one or both of these bodies in the urine increases the gravity of the prognosis, still they are very often encountered without the occurrence of any specially adverse conditions. In the less severe forms of acute diabetes, a judicious modification of the dietary often frees the urine, at any rate for a time, from their presence. For example, the urine of a young man when admitted into hospital contained 38 grains of sugar to the ounce along with a considerable quantity of diacetic acid and acetone, which speedily disappeared under the influence of a restricted diet, the percentage of sugar coming down more slowly. During several months stay in hospital the diacetic acid did not return unless large quantities of fat were given, the sugar meanwhile diminishing and eventually disappearing altogether. He was discharged, but returned in a few weeks with 40 grains of sugar to the ounce and more diacetic acid than before; this again disappeared with a carefully adjusted diet. Even in the severe form of diabetes, diacetic acid may persist in the urine for months before the threatened coma comes on.

In the last stage of many chronic diseases, especially in chronic uræmia, the odour of acetone is given off by the patient, usually

¹ *Pathol. des Harnes*, 1903.

² *Wiener klin. Wochenschr.*, 1901.

³ *Deutsch. Arch. f. klin. Med.*, 1903.

mingled with a blend of butyric acid, or of some volatile body which partially disguises the purely ethereal odour of acetone as perceived in diabetic coma. In chronic uræmia the respiration may also resemble that of diabetic coma—slow, deep, and sighing; if the urine is examined, the reactions of diacetic acid and of acetone may be obtained. Saundby¹ records a case of this kind, and the author has seen several in which both diacetic acid and acetone were detected in the urine, and in one case β -oxybutyric acid also. Acetone is often met with in children apart from febrile conditions, especially in gastro-intestinal derangements.

Detection in urine.—The attention of the physician is usually directed to a patient who is excreting an abnormal amount of acetone by the peculiar, ethereal odour that is perceptible in his vicinity, chiefly given off by the lungs. The odour has been variously likened to that of chloroform, of acetic ether, and of American apples. The urine from such a patient may have the same odour.

Legal's test.—To a little of the urine in a test-tube a few drops of a freshly prepared strong solution of sodium nitroprusside are added; the mixture is then alkalisied with a little liquor potassæ, when a ruby-red colour is produced, which pales to yellow. On acidulating with acetic acid, a red or purple-red colour appears, which is permanent for some time, tending to become blue after many hours standing. This test also reacts with aldehyde. By alkalisising with ammonia (without subsequent acidulation), le Nobel avoids the reaction with aldehyde, the coloration being developed very gradually. For clinical purposes Legal's test is the most satisfactory, but it is not so delicate as the following test.

Lieben's test.—This test is founded on the property possessed by several substances, which contain the CH_3 group, of forming iodoform when added to an alkaline solution of iodine. It may be performed (a) with the urine itself, but it is preferable to use (b) a distillate obtained from the urine.

(a) To a test-tube half filled with the urine half a dozen drops of a strong aqueous solution of iodine in potassium iodide are added, and then as much solution of potassium hydrate as is necessary almost to remove the colour imparted by the iodine. On heating, the urine becomes turbid from the formation of crystals of iodoform, and gives the well-known odour of that substance. When the amount of acetone is small the mixed liquid should be boiled for a few seconds, and then, on holding the test-tube in a stream of cold water, the iodoform is precipitated.

¹ *Renal and Urinary Diseases*, 1896.

(b) About 300 c.c. of the urine, slightly acidified with a few drops of hydrochloric acid, are placed in a distillation-flask furnished with a condenser which must be kept well cooled. As the acetone is very volatile most of it passes over in the first 20 c.c. of distillate, which is treated as (a). The crystals of iodoform, when examined under the microscope, appear either as rosettes or as six-sided tablets like cystin crystals.

In the process of distillation, any diacetic acid that is present in the urine is converted into acetone, and appears as such in the distillate.

Estimation.—The best results are obtained with the Messinger¹-Huppert² process, which consists in adding a known quantity of iodine in alkaline solution to a distillate from the urine, and then, by titration, ascertaining how much of the iodine remains unconverted into iodoform. To 100 c.c. of the urine 2 c.c. of 50 per cent. acetic acid are added, and the urine is then distilled into a receiver fitted with an extra condensation bulb, until five- or seven-tenths has passed over; the residue, diluted with water, is tested for any remaining acetone by distilling a small quantity and testing the distillate by Lieben's test. If all the acetone has not passed into the first distillate, the distillation of the residue must be resumed until it is free from acetone. The total distillate, acidulated with 1 c.c. of 12 per cent. sulphuric acid, is redistilled, and is received into a larger flask fitted with an extra bulb to ensure condensation of all the acetone, the volatility of which necessitates ice-cooling of the whole condensing system. To the second distillate, about 20 c.c. of N/10 solution of iodine are added, and then some 50 per cent. solution of sodium hydrate until the blue colour disappears and a precipitate of iodoform separates; this needs an excess of the soda solution. The flask is shaken for half a minute and then allowed to stand for five minutes. After this the solution is acidulated with hydrochloric acid, which develops a brown colour indicating that the iodine is in excess; the solution is then titrated with N/10 solution of sodium hyposulphite. As soon as the hyposulphite produces a faint yellow colour a little starch solution is added, and then the addition of the hyposulphite is continued until the blue colour produced by the starch disappears, which is the end reaction. One c.c. of the hyposulphite solution equals 1 c.c. of the iodine solution, or 0.967 milligramme of acetone, so that by subtracting the number of cubic centimetres of the hyposulphite solution used from the number of cubic centimetres of iodine solution used, and multiplying the result

¹ *Berichte d. deutsch. chem. Gesellsch.*, 1888.

² *Loc. cit.*

with the factor 0.967, the amount of acetone present in 100 c.c. of the urine is determined. It is essential that the final distillate should contain neither phenol, ammonia, formic acid, nor nitrous acid. Phenol is kept back from the first distillate by the acetic acid, ammonia from the second by the sulphuric acid, and nitrous acid by treating the first distillate with calcium carbonate should potassium iodide and starch-paper be turned blue when dipped into the distillate. Formic acid is removed by the same means.

PROTEIDS.

THE proteid substances that occur in urine may be classified as (a) Serum proteids: *serum albumin*, *serum globulin* or *paraglobulin*, and *fibrin*. (b) Compound proteids: *nucleo-albumin*, *chondro-albumin*, *taurochol-albumin*, and *mucins*. (c) Proteolytic products: *albumoses* and *peptone*.

With the exception of fibrin, all proteid bodies that appear in urine are in a state of solution: albumin and the later products of proteolysis are soluble in water; globulin, the compound proteids, and the earlier products of proteolysis are insoluble in water, but are held in solution by the urinary salts. As colloidal bodies proteids are not diffusible through membranes, consequently they can be separated from saline matter by dialysis; the salts (which are readily diffusible) pass through vegetable parchment into distilled water, whilst the proteids are left behind. When the salts are thus removed from urine, the globulin and the other proteids present that are insoluble in water are spontaneously precipitated. Under conditions that are determined by the reaction of the urine and by the amount of saline and other substances it contains, most of the urinary proteids are coagulable by heat. Secondary albumoses and peptones excepted, the urinary proteids are precipitated by nitric acid; with most of the proteids, except albumin, the precipitate thus formed is soluble with the aid of heat. By saturating urine with certain neutral salts, and in the case of some by half saturation, the proteids present can be fractionally precipitated. Fibrinogen is precipitated by half saturating its solutions with sodium chloride, and hetero-albumose almost entirely by full saturation with the same salt; globulin and the compound proteids by saturation with magnesium sulphate, and albumin along with all other proteids except some deuterio-albumoses and peptone, by saturation with ammonium sulphate. Halliburton¹ and others have shown that when proteids are thus precipitated at the ordinary temperature they are not coagulated; such precipitates can be dissolved in suitable media, the solutions giving the characteristic reactions of the proteid.

¹ *Journ. of Physiol.*, 1884.

All proteids have been regarded as being lævo-rotatory. Recently, Gamgee and Jones¹ have shown that the nucleo-proteid of the pancreas is dextro-rotatory $[\alpha]_D = +97^\circ 9$, and the nucleo-histon of the thymus $+37^\circ 58$. When a nucleo-proteid by loss of a fraction of the albumin molecule becomes of the "nucleine" type its specific rotation is increased. Gamgee considers it probable that all nucleo-proteids are dextro-rotatory. Proteids give a variety of colour reactions with a vast number of reagents. In the clinical examination of urine recourse to the colour-reactions of proteids is usually limited to what is known as the biuret-test, in which the urine is made strongly alkaline with potash or soda, and then a drop of a very dilute solution of copper sulphate is added; by gently agitating the test-tube the copper salt is diffused through the upper stratum of urine, when, if albumin only is present, a violet coloration results; if albumose or peptone is present the colour is rose-red, which is the true colour of the biuret-reaction.

SERUM PROTEIDS.

ALBUMIN.

Albumin is soluble in water and in weak saline solutions; it is also soluble in saturated solutions of some salts, such as sodium chloride and magnesium sulphate; it is insoluble in alcohol. Halliburton² states that blood serum contains three varieties of albumin, which are distinguished by different coagulation-temperatures ranging from 73°C. to 84°C. ; and also that traces of acid lower, and of alkali raise, the temperature of coagulation. According to Brunton and Power,³ the coagulation-temperature of albuminous urine varies between 55.6°C. and 82.2°C. ; it is raised by the presence of urea. By the action of acids and of alkalies albumin forms albuminates, being converted respectively into acid albumin or syntonin, and alkali albumin, both of which are insoluble in water, but are soluble in dilute saline solutions, such solutions not being coagulated by heat. Albumin is precipitated by excess of a mineral acid, but not by acetic acid; more hydrochloric acid is required to produce precipitation than is the case with nitric acid. (Huppert.⁴)

GLOBULIN.

Globulin is insoluble in water and in alcohol, but is soluble in dilute saline solutions; such solutions are coagulable by heat and by

¹ Hofmeister's *Beiträge z. chem. Physiol.*, 1903.

² *Journ. of Physiol.*, 1884. ³ *St. Bartholomew's Hosp. Reps.*, 1887.

⁴ *Loc. cit.*

PROTEIDS.

acids. The precipitate caused by the addition of a few drops of acetic acid is readily soluble in excess. Globulin is converted by acids and alkalis into albuminates. Like albumin, globulin is divisible into several varieties, which are distinguishable by fractional precipitation with ammonium sulphate. Hammarsten's fibrinoglobulin is precipitated by 26 per cent. saturation, euglobulin by 28 per cent., and pseudoglobulin by 35 per cent. saturation.

ALBUMINURIA.

Albuminuria denotes the presence in the urine of serum albumin accompanied by a varying proportion of globulin.

Normal urine contains a minute trace of albumin, which, however, is too small to be detected by any of the ordinary clinical methods of testing; therefore, when the presence of albumin can be recognised by the direct application of any of the usual clinical tests the condition of the urine is abnormal, and constitutes albuminuria. This does not mean that the presence of an amount of albumin in the urine, which is capable of demonstration by clinical methods, is necessarily due to a pathological condition; still less does it prove that the kidneys are diseased. Clinical experience shows that albuminuria may occur under a variety of conditions, some of which are obvious, others are not, in which no other ascertainable deviation from the assumed healthy standard can be detected. It is a well-established fact that transient albuminuria may occur in a healthy man after prolonged and severe exercise; now although albuminuria thus produced cannot be regarded as affording evidence of the normal occurrence of albumin in the urine, neither can it be accepted as a manifestation of disease. It is also much more frequent than is generally known for the urine of people in perfect health to contain albumin for short periods without any ascertainable cause. Such people may, and often do, live to advanced age; some with occasional returns of the albuminuria, others, so far as it is possible to ascertain, without any recurrence; in both classes this one symptom is the sole indication that there is anything amiss with the renal function. In some individuals there can be little doubt that the renal epithelium, without manifesting any other deviation from the normal, is inherently less resistant to the albumin-molecule than renal epithelium in general. Then come those cases described as "functional albuminuria," in which, although the abnormal condition is often of considerable duration, kidney disease is rarely developed. Intermittent functional albuminuria sometimes occurs in families, several members of which are subject to irregular

periods of albuminuria without any other symptom. When albumin is only occasionally and erratically present in the urine, that which is passed shortly after mid-day is most likely to yield positive evidence to tests. Although in these cases the amount of albumin is usually insignificant, the occurrence of as much as 0.4 per cent. is recorded.

Functional albuminuria.—Several varieties of "functional" albuminuria are described. Moxon¹ recorded a number of cases of this type, to which he gave the name *albuminuria of adolescents*; many of the patients suffered from feeble circulation, and their urine contained crystals of calcium oxalate; in one case an epithelial cast was occasionally observed. Pavy² describes a form of functional albuminuria which he calls *cyclic albuminuria*; in this condition the early morning urine is free from albumin, which begins to appear about ten or eleven o'clock; it declines in the afternoon and by evening has disappeared. Although, usually, cyclic albuminuria occurs in youth, Pavy saw one case aged forty-nine years. Charrin³ relates the case of a healthy girl, aged eleven years, whose morning urine contained at the most only a trace of albumin; the albumin gradually increased during the forenoon and reached its maximum after the mid-day meal, after which it disappeared until at bedtime it was all but gone. Between three and half-past four in the afternoon, not only did the maximum of the albuminuria subside but the toxicity and the molecular concentration of the urine were also lessened; at the same time the body temperature, the intake of oxygen, and the blood pressure were all reduced.

To another variety the designation *postural albuminuria* is given, the presence of albumin being determined by the more or less upright posture of the trunk. The following case observed by the author illustrates this form of functional albuminuria: A healthy young man, aged thirty years, was found to have albumin in the urine; it was also found that the albumin never appeared in the urine that was passed on rising in the morning. He was told to have his breakfast in bed, and to remain there several hours subsequently; but the urine he voided contained no albumin until he had been up for a time. A few days after he remained, until late afternoon, in bed, where he had breakfast and lunch, and whilst in the horizontal posture he made vigorous movements of arms, legs, and trunk at repeated intervals; still the urine remained free from albumin. He then got up and sat quietly reading for two hours, when the first urine he passed was albuminous. The experiments were repeated, always with the same results, and if the subject of

¹ *Guy's Hosp. Reps.*, 1878.

² *The Lancet*, 1885.

³ *Journ. de Physiol.*, 1901.

the experiments remained in bed the entire twenty-four hours, taking his usual meals, the urine remained free from albumin. The amount of albumin was small, never exceeding 0.07 per cent.; no casts were ever found, nor any crystals of calcium oxalate, nor of uric acid. The specific gravity of the urine passed at various times, and the percentages of urea, showed no material variations, nor was there anything abnormal about the urine except the albumin. Pulse-tracings afforded no evidence of reduced arterial tension. Herringham¹ records instances of a similar kind and attributes the albuminuria to the weight of the blood on the renal veins.

The author recently observed a very exceptional case: a healthy man, aged forty-two years, whose urine six months previously contained no albumin, was found to yield a trace early in the morning and none during the rest of the day; this condition continued for many weeks, during which the day urine was always free from albumin whilst that passed on rising in the morning always showed a trace.

Alimentary albuminuria constitutes another type of functional albuminuria of which two varieties are met with. The following case illustrates one variety: The urine of a girl, aged eighteen years, who was for some time in hospital under treatment for anæmia, was observed to contain a small amount of albumin; that which was voided before breakfast was free; after breakfast it contained albumin, although the patient remained in bed. The albumin only appeared after breakfast, which consisted of bread and butter and coffee with milk; if the meal was postponed, the urine remained free from albumin. In this variety, the simple ingestion of food is sufficient to cause albuminuria.

The other variety of alimentary albuminuria is due to the ingestion of white of egg, which under normal conditions is changed in the intestinal cells before it reaches the blood; if, however, more white of egg be absorbed than the cells can deal with, a portion of it reaches the blood unchanged, whence it is at once removed by the kidneys. In some individuals the intestinal cells appear to be unable to deal with more than a very limited amount of egg-albumin; consequently, in them, this variety of alimentary albuminuria is easily produced.

Surface chills, such as are caused by cold baths, may produce transient albuminuria; four such cases, occurring in young students, are recorded by Johnson.² In a number of thin, weakly subjects, Rem-Picci³ found that cold baths below 50° F., lasting for three

¹ *Brit. Med. Journ.*, 1891.

² *Trans. of Clin. Soc.*, 1874.

³ *Bolletino d. R. Accad. di Roma*, 1901.

to four minutes, caused albumin to appear in the urine within ten minutes; in twenty-four hours it had all disappeared. When the temperature of the water was above 70° F. albuminuria was not produced.

Occasionally it occurs that albumin is accidentally discovered once only in the urine of a healthy man; in the absence of vaso-motor disturbance, or of other obvious cause, it is probable that the fugitive presence of some unknown and unsuspected toxin in the blood had transiently deprived the renal epithelium of its power to resist the passage of albumin. It is known that the excretion by the kidneys of some forms of altered albumin, such as urinary peptone (deutero-albumose), may temporarily damage the epithelium to such an extent as to produce a slight attack of albuminuria.

In most cases of simple albuminuria, renal casts are usually absent from the urine, or, if present, they are limited to the occasional appearance of a hyaline cast. It is to be observed, however, that when the blood pressure has been suddenly disturbed, as after prolonged, arduous exercise, and possibly in consequence of the rapid and excessive formation of certain excretory products which derange the tubular epithelium during their elimination, casts may be numerous for a short time without being indicative of more than temporary derangement.

PATHOLOGICAL ALBUMINURIA.

The conditions which give rise to this type of albuminuria comprise:—All the various forms of nephritis; the damage done to the renal epithelium by the passage through it of toxic substances present in the blood, either autogenous—as in diphtheria or exogenous—as in poisoning by cantharides, phosphorus, mercuric salts, and other poisons; the presence of a renal calculus or of pyelitis; general venous obstruction, such as occurs in some forms of heart disease and occasionally in other conditions; the pyrexial stage of fevers and of many other acute diseases. Certain diseases are liable to be accompanied by albuminuria; such are apoplexy, epilepsy, purpura, and some forms of anæmia; gout is often associated with albuminuria, due to the presence of contracting kidney. During gestation and parturition albuminuria is not uncommon; it is probably due to excess of work thrown on the kidneys by the placental circulation, or by the foetal products derived from it, which irritate the renal epithelium; in parturition the venous congestion produced by the intense straining probably has an influence. Albumin may appear in the urine after mechanical injury to the

kidney, caused by a contusion, or a blow, without the occurrence of hæmaturia. (Edlefsen,¹ Engel.)² Suppurative diseases of the lower urinary tract, such as cystitis and urethritis, give rise to albuminuria; also hæmorrhage from the same parts and paroxysmal hæmoglobinuria. It is to be borne in mind that in women albuminuria may be wrongly assumed on account of the urine being voided just before the visible commencement of a menstrual period, or before its absolute cessation; in either case the urine may present no obvious appearance of being contaminated with blood.

The amount of albumin that is present in albuminuria is usually below 1 per cent., and it rarely exceeds 2 per cent.; very exceptionally it may reach 5 per cent.; in pyrexial (? toxic) albuminuria, when uncomplicated, it is limited to a trace. When much albumin is present, the froth which is formed by shaking the urine, or by pouring it from one vessel to another, persists longer than is the case with normal urine. Uroerythrin is usually absent in severe albuminuria, as is also urobilin, of which, however, there may be a small amount.

In albuminuria, the proportion of serum globulin which accompanies the albumin is usually small and bears no relation to their relative proportions in the blood. The urinary ratio undergoes considerable variation, being generally the highest during the night and lowest in the morning; under exceptional conditions, globulin alone has been found in the urine. The molecular weight of globulin is calculated to be about 16,000, whilst that of albumin is only about 5000 (Schulz³ determines it at 5100), a relation that is held by Halliburton,⁴ Brodie,⁵ and others, to account for the fact above stated that albumin is much more abundantly present than globulin in albuminuric urine. By means of Martin's method of filtration through gelatine, which by variations in its density can be made to act as a more or less porous filter, Brodie shows that on careful adjustment of the gelatine mass, proteid bodies pass through with more or less ease in accordance with their known molecular dimensions. The small molecule of albumose passes through a mesh by which the larger molecule of albumin is arrested; in its turn albumin passes where globulin is held back. On the other hand, hæmoglobin, which, according to Schulz, has a molecular weight of 14,800, is most difficult to keep back. Brodie corroborated these results by experiments made with the excised kidney, and expresses

¹ *Münchener med. Wochenschr.*, 1902.

² *Berliner klin. Wochenschr.*, 1903.

³ *Die Grösse des Eiweissmoleküls*, 1903.

⁴ *Trans. Path. Soc. Lond.*, 1900.

⁵ *Ibid.*

the opinion that the occurrence of albumin alone in urine would indicate a practically complete membrane of the kidney-tubules, and that an increased proportion of globulin points to imperfection of the tubule walls by actual loss of cells. Still there are difficulties in the way of regarding the transmission of colloidal substances through the renal epithelium solely in the light of their molecular constitution; for example, the easy transmission of the large hæmoglobin molecule, the erratic appearance of excessive amounts of globulin, and the remarkable fact, to which Klemperer¹ directs attention, that the urinary pigment, urochrome, although of large molecular size, is an ordinary constituent of normal urine.

Many attempts have been made, by Maguire² and others, to utilise the varying ratio in urine of albumin to globulin as an aid to prognosis, the proportion being formulated as the "proteid quotient," or $\frac{\text{albumin}}{\text{globulin}}$. The quotient, however, varies so capriciously, and between such excessively wide limits—according to Paton³ from 0.6 to 39—as to deprive it of much prognostic value. Such variations appear, not unfrequently, in simple, mild cases of albuminuria; they occur without any perceptible alteration in the clinical aspect of the patient, and they come and go at such short intervals as to make it difficult to believe that they are dependent on material changes in the secreting surface of the kidney. At the same time, it is to be observed that in the advanced stage of many cases of Bright's disease, a marked and persistent increase in the proportion of globulin in the urine is distinctly an unfavourable sign, and is probably due to denudation of some of the tubular walls: under these conditions the excess of globulin usually indicates that the end is near. The presence in urine of an excess of globulin is often obvious to the unaided eye. When a column of urine that contains much globulin is viewed from above, as in looking down on a full urine glass, a peculiar opalescence may be observed, as though the urine were slightly gelatinous; this is most likely to occur in urines that are poor in salts. Bramwell and Paton⁴ record a unique case in which the urine spontaneously deposited rhombic crystals of globulin; in this case the urinary proteids were chiefly represented by globulin; on one occasion, out of a total of 2 per cent., 1.92 per cent. consisted of globulin. After death, no waxy, fatty, nor other obvious changes were found in the kidneys.

¹ *Congres. f. inn. Med.*, 1902.

² *The Lancet*, 1886.

³ *Brit. Med. Journ.*, 1890.

⁴ *Lab. Repts. Royal Coll. Physicians Edin.*, 1892.

FIBRIN.

Fibrin is present in the urine when hæmorrhage from any part of the urinary tract has occurred; it is also met with in acute, toxic inflammation of the mucous membrane of the lower tract and, very exceptionally, is derived from the secreting surface of the kidney, either in consequence of the presence of a renal calculus, of amyloid degeneration, or of an abscess. Except as an accompaniment to hæmaturia, fibrinuria is of rare occurrence, especially when it takes the form of a colourless exudation from the renal tubules. A short time after being voided, in urine that contains an obvious amount of fibrin, yellowish or greyish-white gelatinous masses are seen; if a large amount of fibrin be present the whole of the urine is converted into a gelatinous mass, sometimes presenting the appearance of fine threads traversing it in all directions. In such cases the urine may clot like a serous fluid, when it may be detached in a mass from the containing vessel; if allowed to stand for several hours the clots disappear, being dissolved in the urine. Klein¹ records an exceptional case of this kind, in which a man, aged fifty-two, passed urine that contained large amounts of albumin; after the urine had stood for a time, elongated clots, greyish-white in colour, and from half an inch to four inches in length, some being an inch thick, settled in the lower stratum. At these times the urine was alkaline and poor in phosphates, but it contained a high percentage of albumin. After death the kidneys were found to be undergoing waxy degeneration, and their tubules contained hyaline casts of fibrin. Löstorfer² records the case of a woman, aged forty-nine, who passed large fibrin-clots in clear, pale urine, in which there was a considerable amount of albumin. On section, the urinary passages were found to be intact; the kidneys showed indications of inflammatory processes along with amyloid degeneration. In a case of chronic pyelitis, the pyuria on several occasions was replaced by light-coloured, clear urine, which contained large quantities of fibrin. When poured from one vessel to another the urine flowed in clotted masses, which were not perceptible when it was standing; the clots gave the reaction of fibrin. After standing for a few days the clots spontaneously dissolved, and then when decanted the urine flowed like oil.

COMPOUND PROTEIDS.

Apart from mucin, three compound-proteids, which possess mucinoid characteristics, have been found in urine, namely—*nucleo-albumin*, *chondro-albumin*, and *taurochol-albumin*.

¹ *Wiener klin. Wochenschr.*, 1896.

² *Ibid.*, 1903.

Nucleo-albumin, a combination of nucleinic acid and albumin, is derived from the nuclei and protoplasm of cells; it contains about 1.5 per cent. of phosphorus, the proportion varying with the amount of nucleinic acid present, which, in its turn, is determined by the kind of cell whence the nucleo-albumin emanates. A variety of nucleo-proteid, named **nucleo-histon**, first obtained by Kossel and Lilienfeld¹ from the thymus, has been found in the urine from cases of pyelo-nephritis, purulent phthisis, Hodgkin's disease, chronic nephritis, and jaundice. (Jolles.²)

Chondro-albumin is formed by a combination of chondroitin-sulphuric acid and albumin, and in general characteristics closely resembles the nucleinic combination.

Taurochol-albumin is a combination of taurocholic acid and albumin. According to Mörner,³ who was the first accurately to determine the constitution of these bodies, chondro- and nucleo-albumins are always present in urine, the former in the largest amount; whereas taurochol-albumin only rarely appears, except in icteric urines.

Nucleinic and chondroitin-sulphuric acids possess the property of precipitating, from acid solutions, serum albumin and less perfectly albumoses, the combinations thus formed being soluble in alkaline and saline liquids. By virtue of its saline constituents, normal urine, notwithstanding its acid reaction, is able to retain a certain amount of these compound proteids in solution; but if the urine is poor in salts, for example, after dialysis, or if it is freely acidulated by the addition of an acid, any compound proteids that are present are at once precipitated.

The compound proteids do not always contain the same proportion of albumin, and consequently their properties and reactions vary; when the proportion of albumin is large, the reactions yielded to the ordinary clinical tests may easily be misinterpreted, and accepted as evidence of the presence of pathological albuminuria. Misinterpretations of this kind have led to the publication of extraordinary statements as to the frequency of albuminuria in healthy people. From the clinical standpoint, it is absolutely essential to distinguish between true albuminuria, in which, owing to some fault in the renal epithelium, serum albumin has passed directly from the blood into the urine, and a condition due to the presence in it of a compound proteid chiefly derived from the lower urinary passages. The compound proteids have been found in the urine from cases of

¹ *Zeitschr. f. physiol. Chem.*, 1895.

² *Zeitschr. f. klin. Med.*, 1898; *Zeitschr. f. physiol. Chem.*, 1898.

³ *Skand. Arch.*, 1895.

acute infectious disease, pneumonia, leucocythæmia, chlorosis, acute gastric catarrh, various diseases of the liver, tuberculosis, catarrhal affections of the bladder and urethra, irritation of the pelvis of the kidney from the presence of a calculus, in the early stage of nephritis, and in jaundice. The toxins formed in the course of many general diseases, such as influenza, not infrequently irritate the renal epithelium short of the production of albuminuria, but enough to cause the appearance of a compound proteid in the urine. When the kidneys are more profoundly affected, this primary result of toxic irritation may sometimes be observed immediately before the occurrence of true albuminuria.

The ambiguity which surrounds the reactions attributed to the compound proteids has led to the assumption that most of the proteid substances in urine which are precipitated by acetic acid consist of one of the globulins, or of fibrino-globulin, with possibly an admixture of nucleo-albumin. In blood serum, Freund and Joachim¹ found a phosphorus-containing body which falls within the precipitation limit of euglobulin, and which they consider to be nucleo-globulin. Rostoski² finds that the proteids in pathological urines which are precipitated by acetic acid, have precipitation limits that correspond to fibrino-globulin and euglobulin; he also finds that a small amount of nucleo-proteid may be present. The precipitation limit of those proteids that are thrown down on the addition of acetic acid may be determined by taking 10 c.c. of a solution of the proteid and ascertaining how many cubic centimetres of a saturated solution of ammonium sulphate are required to precipitate the proteid. Matsumoto³ found that the precipitation limits of the globulins are much higher than those of nucleo-albumin which lies between 0.1 to 0.8 as the lowest limit, and between 1.6 to 2.2 as the highest; he also found that the precipitation limits of the urinary proteids that are precipitated by acetic acid are much nearer those of the globulins than of nucleo-proteid, and on these grounds infers that the proteid substances in question are probably of the nature of fibrino-globulin, though occasionally nucleo-albumin also may be present.

MUCINS.

The colloid proteids of this class do not contain phosphorus; on being boiled with dilute mineral acids mucins yield a substance—glucosamine—which reduces Fehling's solution, but is not fermentable. Like other compound proteids, mucins differ in some of their

¹ *Zeitschr. f. physiol. Chem.*, 1902.

² *Sitz. bericht. d. phys. med. Gesellsch.*, Würzburg, 1902.

³ *Deutsches Arch. f. klin. Med.*, 1903.

characteristics in accordance with the sources whence they are derived ; to what extent the mucinous substance that appears in urine is to be regarded as a true mucin is an open question. Mörner¹ calls it urine-mucoid ; the mucoids being a class of mucinous bodies which yield reactions that differ from those given by true mucins—they are not precipitated from alkaline solution by acetic acid, or if so, are readily soluble in excess. Mucin is the chief substance that gives the viscid character to mucus. The delicate, translucent cloud, or nubecula, deposited by healthy urine, consists of mucus with epithelial cells and leucocytes entangled, and represents the normal secretion of the mucous membrane of the urinary passages ; so that urinary mucin, or mucoid, exists partly in solution in the urine and partly in suspension in the nubecula. The mucous membranes in different parts of the body secrete mucus that contains varying proportions of mucin ; when the mucin is scanty it is often replaced by a nucleo-proteid. In some of the lower animals “biliary mucin” is exclusively composed of taurochol- or of nucleo-albumin ; in human beings, according to Hammarsten,² it also contains true mucin.

As with the other compound proteids in urine, the amount of mucin is increased by any conditions which give rise to irritation of the mucous membrane of the urinary tract. In simple, non-specific urethritis, or when the urine contains abnormal substances of an irritating nature, or even when it is merely very concentrated, and has a strong acid reaction, a varying excess of mucinoid substances will be present.

PROTEOLYTIC PRODUCTS.

Albumoses represent intermediate stages in the hydrolysis of proteids to peptones, the ultimate product of proteolytic action. The proteids of food are mostly absorbed in the form of albumoses and peptones which, under normal conditions, are modified as they pass through the epithelial cells of the intestinal walls, so that they reach the blood in another and at present undetermined form ; the consequence is that, under normal conditions, neither albumoses nor peptones are present in the blood. If the smallest amount of any of these substances reaches the blood, it is dealt with as a foreign body, is at once removed by the kidneys, and albumosuria or peptonuria results. The distinction between the different albumoses and peptones is artificial and is determined by the respective behaviour of these substances towards certain chemical reagents.

¹ *Skand. Arch. f. Physiol.*, 1895.

² *Jahresbericht d. Thier.-Chem.*, 1893.

As the result of the investigations of Kühne,¹ the products of the digestion of proteids have been classified as primary and secondary albumoses and peptone: in urine hetero-albumose of the first group, and deutero-albumose of the second, are the types most commonly met with; proto-albumose less frequently. Like globulin, hetero-albumose is insoluble in water, or nearly so; deutero-albumose readily dissolves in water. Both are precipitated by saturating their solutions with ammonium sulphate, but deutero-albumose only after successive saturations in acid and in alkaline solution; hetero-albumose is almost completely precipitated by saturation with sodium chloride, whilst deutero-albumose is not. Nitric acid precipitates hetero-albumose, but not deutero-albumose. In neutral solution all albumoses are precipitated by alcohol; the precipitate of deutero-albumose is not coagulated, that of hetero-albumose is partially so; from acid and alkaline solution alcohol does not precipitate any of the albumoses.

So far for the actual products of proteid digestion as formed in the alimentary canal. The analogous proteids found in urine are scarcely ever, if ever, derived from the intestinal tract; they are independently formed (*a*) by the action of micro-organisms on inflammatory exudation products, or on the tissues themselves; and (*b*) concurrently with the formation of certain neoplasms, especially multiple myeloma.

(*a*) PEPTONURIA.

In this condition the proteid substance in the urine takes the form of proto- or deutero-albumose, the nearest proteolytic products to peptone, and its presence constitutes what was formerly regarded as true peptonuria, and for which the term "peptonuria" is still retained. As justification for the retention of the term "peptonuria," Huppert gives the name "urinary peptone" to the substance in urine which resembles deutero-albumose. True peptone, the end product of physiological digestion, is not formed in the organism apart from the proteolysis that occurs in the digestive tract; and the passage of unchanged peptone from the intestine to the blood is of very doubtful occurrence. Halliburton,² whilst stating that destruction of the columnar epithelium of the intestinal wall, which synthesises the peptone into albumin and globulin, would be a likely cause for the appearance of peptone in the urine, also states that he has never met with a well-attested case of the kind, although in severe intestinal diseases the epithelium is certain to suffer. As

¹ *Zeitschr. f. Biolog.*, 1884.

² *Trans. Path. Soc. Lond.*, 1900.

far as our present knowledge goes, it may be accepted that the occurrence in the urine of true peptone, derived from the digestive tract, is most exceptional if it occurs at all (Stadelmann,¹ Devoto,² Senz,³ and others).

The diseases in which albumoses (urinary peptone) have been found in urine comprise: empyema, lobar pneumonia, disintegrating tubercular deposits as in purulent phthisis, malignant growths, especially of any part of the digestive tract and also in other tissues. In a number of septic diseases as septicæmia, variola, diphtheria, scarlet fever, measles, enterica, typhus, malaria, erysipelas, acute rheumatism. In various pathological conditions affecting the liver: acute atrophy, acute phosphorus poisoning, alcoholic and cardiac enlargement; and in involution of the puerperal uterus.

In some of these cases it is permissible to doubt the accuracy of the statements; for, as elsewhere pointed out, some urinary proteids are easily confused with others, and too much importance has been attached to the biuret reaction as a test for albumoses [see *Biuret reaction*]. Even admitting their accuracy, the occurrence of peptonuria in many of the diseases named can only be fortuitous, as a vast number of comparative observations have been made with negative results.

A phosphorus-free proteid—**histon**—has been found in the urine, in some of the above-named conditions, which bears a certain resemblance to urinary peptone. Histon was first obtained by Kossel⁴ from the nuclei of the red blood-corpuscles of the goose; it is supposed to be a decomposition product of nucleo-histon.

(b) ALBUMOSURIA.

In this condition, the proteid body which appears in the urine was first described by Bence Jones,⁵ and is still known by his name. Its appearance in urine is closely associated with diseases of the bones, which implicate the marrow, especially multiple myeloma. After a varying period of increasing debility, the patient complains of pains which are often localised over certain bones, where enlargements may or may not be perceptible. The bones become soft and brittle; spontaneous fracture may occur, and distortion of the bones of the spine, with kyphosis. After death, which usually occurs within two years after the commencement of the symptoms, the

¹ *Untersuch. u. Peptonurie*, 1894.

² *Zeitschr. f. physiol. Chem.*, 1891.

³ *Ueber Albumosurie u. Peptonurie*, 1891.

⁴ *Zeitschr. f. physiol. Chem.*, 1884.

⁵ *Philosoph. Trans. Roy. Soc.*, 1848.

bones are found to be rarefied and softened, the marrow being replaced by a red jelly-like mass; the condition resembles lymphoma, or round-celled sarcoma. Almost all the recorded cases have occurred in men.

In the early stage of the disease, before any definite symptoms reveal themselves, the urine possesses peculiar characteristics. It may be clear when passed, or, as in a case reported by Bradshaw,¹ it may be turbid like milk and water; in this case it deposited a copious, white, amorphous sediment, which, when separated and dried, formed a glue-like mass. When clear the urine is viscid, almost syrupy, and if shaken it produces a long-lasting froth. Whilst the descriptions given of Bence Jones's proteid are to a certain point concordant, they display various discrepancies. In some accounts it is stated that the precipitate thrown down on heating is entirely re-dissolved at a higher temperature, 100° C.; but in several cases, as in one recorded by Hutchison,² the precipitate did not fully dissolve when the urine was boiled. Magnus-Levy points out a number of differences in the reactions obtained by various observers; he discusses the unity and the nature of the Bence Jones proteid and comes to the conclusion that the bodies, which have been found in the urine in the cases so far described, are identical. Amongst other reactions, Magnus-Levy³ obtained from the Bence Jones proteid all the known proteolytic products of proteid digestion except hetero-albumose, and on this and other grounds he concludes that it is not an albumose, but that it is a primary cleavage product of true albumin. Both Magnus-Levy, and Grutterink and Graaff,⁴ obtained proteid crystals from the urine which contained the Bence Jones proteid. Hæmoglobin, the globulin discovered by Bramwell and Paton, and the Bence Jones proteid, are the only proteids found in urine that have been obtained in the crystalline form.

The inter-relation between the occurrence of the Bence Jones proteid in urine and the development of myeloma and kindred diseases of the bone and bone-marrow has not yet been explained; but as the urinary symptom usually precedes the other manifestations of the disease, the discovery of this proteid in the urine is of serious import, and demands a careful investigation of the general condition of the patients in whom it occurs. Exceptionally, as in one of three cases recorded by Anders and Boston,⁵ the albumose may cease to appear in the urine some weeks before death takes place.

¹ *Trans. Path. Soc. Lond.*, 1900.

² *Ibid.*, 1900.

³ *Zeitschr. f. physiol. Chem.*, 1900 (with bibliography).

⁴ *Ibid.*, 1901.

⁵ *The Lancet*, 1903.

THE TESTS FOR URINARY PROTEIDS.

Urine that is to be tested for proteids should be free from turbidity. Cloudiness due to urates may be dissipated by gently warming the urine; when due to other substances filtration will remove it; indeed for delicate testing filtration is always advisable. In some urines the causes of turbidity are not removed by passing the urine through an ordinary filter; when dealing with such urines a very close filter-paper, such as Schleicher and Schüll's No. 589 blue ribbon, must be used. Close-textured paper is essentially slow and is best used with pressure; but in clinical work, as only small quantities of the filtrate are required, simple filtration answers the purpose, the passage of the urine being accelerated by pleating the filter. Some kinds of filter-paper yield sufficient vegetable proteid to the filtrate to make it react with the more delicate tests for albumin; if there is any doubt on this point, half an ounce of distilled water should be passed once or twice through the suspected paper and then tested with trichloroacetic acid.

The proteid substances which appear in urine are usually in solution, and consequently are invisible until, either by means of heat or by the addition of chemical reagents, coagulation is produced; the proteid is then revealed by the occurrence of a faint haze, or of a dense cloud, or clotted mass, in accordance with the amount that is present.

SERUM ALBUMIN.

The Nitric Acid Test.—This test is performed in such a manner as to cause a layer of urine to rest on a layer of nitric acid. There are two ways of accomplishing this: (1) Nitric acid is poured into a test-tube to the depth of half an inch; the tube is then inclined to very nearly the horizontal position, and two or three times the volume of urine is gently poured down the wall of the tube, with a pipette if necessary, so that it floats on the acid. This is the most delicate way of applying the test. (2) A test-tube is half filled with urine, and, whilst inclined as before, a little nitric acid is allowed to trickle down the side in such a way as to pass below the urine and form a layer at the bottom of the tube. This is most conveniently accomplished by using a "drop-bottle" with a grooved stopper. Whichever method be adopted, care must be taken not to allow the acid to mix with the urine. The tube is then held in the vertical position and examined. The presence of serum albumin is shown by a whitish deposit, which commences immediately above

the acid. If the percentage of albumin is great, the coagulated layer is rapidly formed and is dense and opaque; if only a small amount be present, a faint haze or opalescence gradually develops, which may be limited to a thin disc on the surface of the acid, or it may spread as a slight haze some distance up the column of urine. When the reaction-limit of nitric acid for the detection of albumin in urine is all but reached, some time elapses before any haze is visible; therefore, a negative result must not be assumed until the urine has stood in contact with the acid for four or five minutes, without visible change. The appearance of the deposit of coagulated albumin differs in accordance with the mode of testing. If, with a small amount of albumin, the urine is added to the acid, a thin well-defined white or opalescent disc appears, resting on the acid; if the acid is added to the urine, a hazy cloud is produced which extends from the surface of the acid some distance upwards, and then fades off into the clear urine.

Occasionally a cloud forms in the urine higher up in the tube, a layer of clear urine being interposed between the cloud and the acid, or, if serum albumin be present, between the cloud and the layer of coagulated albumin which rests on the acid. This upper cloud indicates the presence of nucleo- or chondro-albumin, or of mucin (*q.v.*). Here again the mode of applying the test to some extent determines the appearances produced. If the urine is added to the acid, the precipitate of the compound proteid appears as a well-defined disc about a centimetre removed from the surface of the acid; if the acid is added to the urine, an ill-defined, broad cloud appears, much higher up in the column of urine. The contrast afforded by the two methods of testing is strikingly displayed when small amounts of both serum albumin and a compound proteid are present in the urine. When the urine is added to the acid, two closely approximating, narrow, and well-defined discs, with a thin layer of clear urine between them, appear within a centimetre of the surface of the acid. When the acid is added to the urine a diffuse cloud of coagulated albumin extends some distance above the acid, and higher up appears a broadish belt with margins not sharply defined, which represents the compound proteid.

Clear, concentrated urines, holding a large amount of urates in solution, often display an immediate precipitate of urates on the addition of an acid; this, as previously explained, is due to interaction between the monohydric and the dihydric phosphates and the biurates, which in this instance is determined by the added acid. The urate-cloud thus formed is yellowish or fawn-coloured, which distinguishes it from the white albuminous cloud; and further, it

begins to form high up, and also along the path taken by the acid as it trickled down the side of the tube, and it rapidly diffuses itself throughout the entire column of urine. The urate cloud quickly disappears on gently warming the urine, and if a second supply of the same urine is diluted with three times its volume of water, and is then tested with nitric acid as before, no precipitation of urates takes place. Very exceptionally, urine in which a urate-cloud is developed by the addition of a drop of acid is amphoteric in reaction, and on boiling another portion of it a faint cloud due to earthy phosphates appears, which dissolves on careful acidulation. In concentrated urines the addition of nitric acid is often followed by the formation of urea nitrate which, being insoluble in liquids that contain free nitric acid, forms a crystalline layer on the surface of the acid; this, it is said, has been mistaken for albumin. The yellowish, semi-transparent, crystalline appearance of the urea salt is amply sufficient to distinguish it from coagulated albumin; moreover, the deposit is dissolved when gently heated. The urine passed by patients who are taking certain resinous drugs, such as copaiba, becomes turbid on the addition of nitric acid; the odour of the urine and the fact that the turbidity disappears on the addition of alcohol, are sufficient to prevent any error of interpretation. In high-coloured urines, the addition of nitric acid determines the appearance, immediately above the acid, of some of the pigmentary bodies elsewhere described, which may materially impede the recognition of the faint haze that is indicative of a trace of albumin; dilution of the urine with one or more volumes of water may possibly remove the difficulty, or Roberts's modification of the nitric-acid test may be used. It consists of a mixture of one volume of nitric acid (1052) and five volumes of a saturated aqueous solution of magnesium sulphate. The urine is floated on the reagent, as with the ordinary nitric-acid test. This reagent precipitates compound proteids and some albumoses as well as serum albumin. On account of its oxidising powers being less than those of pure nitric acid, it does not cause interference by the development of pigments.

The Boiling Test.—A test-tube is filled to two-thirds of its capacity with urine which has been previously ascertained to have a normally acid reaction. If the urine is alkaline, or feebly acid, one drop or more of weak acetic acid is added until the usual acid reaction of urine is obtained. The tube is then held obliquely from the lower end, and the upper part of the column of urine is brought into the flame of a spirit-lamp, the tube being slowly rotated so as to equalise the heat and thus to prevent the tube cracking. The heat is continued until the upper stratum of urine briskly boils, when

the tube, removed from the flame, is held vertically before some dark-coloured surface, such as the sleeve of a black coat. The presence of albumin is indicated by turbidity of the stratum of urine which has been boiled, varying from the faintest haze to a dense cloud. By allowing the light to fall sidewise, or from above, any haze, however faint (provided that the urine was perfectly limpid), stands out in contrast with the unboiled, clear portion. If turbidity occurs, a drop or two of acetic acid should be added to the urine and the effect observed. If the cloud disappears, it was due to precipitation of earthy phosphates; if it persists, it is probably due to albumin. Even to urine that shows no turbidity on boiling, a few drops of acetic acid, or of dilute hydrochloric acid, should be added, inasmuch as occasionally serum albumin may be present, although not visibly so until, on the addition of the acid, a cloud is developed in the heated layer of urine. It is to be noted that this may occur with albuminous urine (usually high coloured) that gives distinctive evidence of acidity with litmus-paper. The probable explanation is, that various nitrogenous substances, especially urea, have the property of protecting albumin from precipitation when boiled in acid solution, possibly by acting as bases. If to a freely acid solution of albumin increasing amounts of urea are added, a point is eventually arrived at when coagulation no longer takes place on boiling. (Spiro.¹) An example of this ambiguous reaction to the boiling-test will show the necessity when testing urine for albumin of systematically adopting the rule of using the nitric-acid test first, and of invariably adding an acid after boiling, whether a cloud has been produced or not. A specimen of rather high-coloured, clear urine, from a case of typhoid fever, gave a distinctly acid reaction with litmus-paper. Nitric acid, in the cold, gave a good reaction indicative of the presence of albumin, and also, higher up the column of urine and separated from the albumin cloud by a layer of clear urine, a second cloud appeared. Yet this urine when boiled in the usual way showed not the least trace of haze until a little acetic acid was added, when the boiled portion at once became cloudy. On further examination the urine was found to contain 0.06 per cent. of serum albumin, along with a small amount of globulin. The second cloud, caused by the nitric acid, was due to a compound proteid. Urea was present in this urine in such excess as to form in a few minutes a thick cake of urea nitrate crystals over the acid. If urine which gives a precipitate of albumin when tested with nitric acid, and none with heat, is saturated with sodium sulphate, the albumin is thrown down by heat as usual (Delaunay²).

¹ *Zeitschr. f. physiol. Chem.*, 1900.

² *Journ. Pharm. Chim.*, 1899.

It often occurs after boiling unacidulated urine possessing a normal acid reaction, that a very faint albuminous haze is perceptible, which becomes intensified on the addition of a drop or two of acetic acid; this increase in turbidity may or may not be due to serum albumin. In order to obtain some differential indications one or two drops of hydrochloric acid may be added; if the turbidity readily disappears, it is probably due to a compound proteid, and to serum albumin if it persists. Or a clear specimen of the same urine may be treated in the cold with a little acetic acid, the urine being previously diluted with two volumes of distilled water if necessary; should a diffuse haze be produced, which persists on heating, it and the haze which develops in the boiled urine after the addition of the acid are both probably due to a compound proteid and not to serum albumin. If, after treatment with acetic acid in the cold, the urine remains clear, the reduplication of the haze produced by acidulating the boiled urine is probably due to albumin which, previous to the addition of the acid, was protected from coagulation notwithstanding the natural acidity of the urine. Sometimes a negative reaction is given by nitric acid in the cold, and no haze appears after the urine is boiled (the urine having a normal acid reaction); but on the addition of a couple of drops of acetic acid a delicate haze is seen in the heated stratum; in such a case, the results obtained by the subsequent addition of hydrochloric acid, as above described, and by the treatment of another specimen of the urine with acetic acid in the cold, will probably enable a differentiation to be made between a minute trace of serum albumin on the one hand and a compound proteid on the other. It is not to be supposed, however, that these tests will always determine the question when but little more than traces of the substances under examination are present in the urine.

Urine which does not react with nitric acid in the cold, and in which the stratum that has been boiled remains clear after the addition of acetic acid, may be pronounced to be free from albumin so far as clinical investigations are concerned. But an equally decided opinion should not be expressed after a single examination of urine that yields the ambiguous reactions just described. More than one specimen, passed at different periods of the day, should be tested, and if there be any deposit it should be examined microscopically for casts.

SPECIAL REAGENTS.

Trichloroacetic acid precipitates albumoses and compound proteids as well as globulin and serum albumin. It is most advantageously used in the form of a saturated solution, and, as it is

extremely soluble in water, some crystals of the acid should permanently remain undissolved in the solution as evidence of absolute saturation. The solution is used in the same way as nitric acid; the presence of a trace of albumin is indicated by a disc of opalescence immediately over the layer of acid; but unfortunately the compound proteids indicate their presence in the same way. The precipitate of albumin is not dissolved by heat; that due to other proteid substances disappears on warming the urine. (See under *Tests for compound proteids*.)

Salicyl-sulphonic acid precipitates all proteids except peptones. It is used as a saturated solution, a few drops of which are added to some perfectly clear urine in a test-tube; the mouth of the tube is closed with the thumb and the contents of the tube are then mixed by shaking. The presence of albumin is shown by a general opalescence or turbidity; if but a trace is present, the opalescence only appears after the lapse of two or three minutes. On heating, the precipitate of albumin and globulin is coagulated; the other precipitated proteids are dissolved and are reprecipitated on cooling.

Picric Acid Test.—When a saturated solution of picric acid is added to urine that contains albumin, it becomes turbid; alkaline urine requires slight acidulation with acetic acid before adding the picric acid. The turbidity may be due to serum albumin, globulin, compound proteids, albumoses, and alkaloidal bodies, such as quinine, if present beyond a mere trace. On heating the turbid urine all these precipitates disappear except albumin and globulin. With traces of albumin, the best results are obtained by carefully adding the picric acid solution so as to form a layer that rests on the urine, when a faint haze at the plane of contact occurs.

Ferrocyanide Test.—After freely acidulating albuminous urine with acetic acid, and then adding a little of a 10 per cent. solution of potassium ferrocyanide, a cloudiness is produced. If opalescence be caused by the acetic acid alone, it is due to a compound proteid and should be compared with that yielded by another specimen of acidulated urine to which the ferrocyanide is subsequently added; any increase in turbidity, which is not dissipated by heat, is due to albumin. Oliver¹ has modified this test so as to present it in an easily portable form. He uses strips of filter-paper respectively impregnated with ferrocyanide and citric acid. For use at the bedside the reagents are dissolved out of the papers in a little water. Pavy uses pellets of the same reagents.

Spiegler's Test.—This consists of 4 grms. of mercuric chloride, 2 grms. of tartaric acid, 20 grms. of glycerine, and distilled water to

¹ *Bedside Urine Testing*, 1889.

100 c.c. A little of this solution is poured into a test-tube, and some of the urine, previously acidulated with acetic acid, is gently poured down the side of the inclined tube so that it may float on the reagent; the presence of albumin is indicated by a white precipitate. All proteids, except peptones, are precipitated by this reagent.

Tanret's Test.—Prepared by dissolving 1.35 grms. of mercuric chloride and 3.32 grms. of potassium iodide, separately in two amounts of distilled water; the solutions are then mixed and 20 c.c. of acetic acid are added, along with distilled water, to 100 c.c. The test is applied in the same way as with the last test; a white precipitate at the junction of the urine with the reagent indicates the presence of a proteid. All proteids are precipitated except peptones.

Jolles's Reagent.—Mercuric chloride 2 grms., succinic acid 4 grms. and sodium chloride 4 grms. are dissolved in 100 c.c. of water. To 4 c.c. of urine, 1 c.c. of acetic acid, and 4 c.c. of the reagent are added and the mixture is shaken. In a second test-tube, the same volumes of urine and of acetic acid are put, along with 4 c.c. of water instead of the reagent. The two tubes are compared; any excess of turbidity in the urine to which the reagent was added is indicative of albumin.

Millard's Reagent.—This consists of twenty-two parts of a 5 per cent. solution of potassium hydrate, seven parts of glacial acetic acid, and two parts of phenol. The urine is floated on the reagent as with the nitric acid test; all precipitates which are produced, except albumin, disappear on heating.

The relative delicacy and trustworthiness of the tests for albumin.—As regards delicacy, those reagents which admit of being used in the stratum manner—a layer of reagent below or above a layer of urine—have the advantage over those which are used in admixture with the urine. Of the former type, the most delicate are: Spiegler's and Tanret's reagents and trichloroacetic acid; of the latter, salicyl-sulphonic acid and picric acid. The ferrocyanide is among the least delicate of these tests.

As regards trustworthiness, a prolonged experience of all the tests named, and of many others of less importance, has led me to the conclusion that, for routine clinical work, the nitric acid test and the boiling-test are the most trustworthy and the least liable to yield fallacious indications; and further, that they are sufficiently delicate for all clinical purposes. The objection to the reagents of exceptional delicacy is not that they react to mere traces of serum albumin, but that they also react to various other proteids in a manner which makes it difficult to differentiate such reactions from those due to albumin, and that they thus give rise to errors of interpretation.

THE ROUTINE-TESTING OF URINE FOR ALBUMIN.

In all cases which come under clinical observation for the first time the urine should be systematically examined for albumin irrespective of the symptoms and the nature of the disease; the frequency with which albumin is unexpectedly found in the urine of proposers for life assurance who suppose themselves to be in perfect health, illustrates the necessity for an undeviating observance of this rule. In ordinary clinical work, the nitric-acid test should first be used, and if a positive reaction is at once obtained there is usually no need to use further qualitative tests; if no reaction occurs at once the boiling-test should be tried. If neither the nitric-acid test after the lapse of five minutes, nor the boiling-test yields a positive reaction, the absence of albumin, from the clinical standpoint, may be safely assumed. Should further evidence be deemed necessary, the trichloroacetic-acid test may be used; the property of this reagent to give ambiguous reactions with other proteids than albumin and globulin being borne in mind. In carrying out these tests, it is obviously understood that all the previously described precautions, as to method and to interpretation of results, are to be minutely observed. (See also the sections on *Testing for compound proteids and albumoses*.)

THE QUANTITATIVE ESTIMATION OF ALBUMIN.

The exact determination of the amount of albumin held in solution in urine is accomplished by precipitating the albumin by means of a suitable reagent; the precipitate is either dried and weighed, or, by means of Kjeldahl's process, its nitrogen is determined, and the result is multiplied by the factor 6.3, which gives the weight of proteids precipitated. If an equal volume of urine and of a 10 per cent. solution of trichloroacetic acid be boiled and filtered hot, all proteids are precipitated except proteoses and peptones; the precipitate is then dealt with as described above. Precipitation may also be effected by tannin.

For clinical use the above method is too circumstantial; therefore recourse is usually had to simpler, though less accurate, methods. The old plan is to add one or two drops of acetic acid to some of the urine in a test-tube and to boil it. The tube is then allowed to stand in the vertical position for a few hours, when the depth of the deposit of coagulated albumin is compared with the height of the urine and the proportion recorded as one-fourth or one-eighth, as the case may be. An improved gravimetric method, known as Esbach's process, is now in common use.

Esbach's Process.—This consists in throwing down the albumin as a finely divided precipitate in a specially graduated glass-tube. The lower half of the tube has a number of graduations—usually from 1 to 7—which respectively indicate the number of grammes of albumin per litre, or tenths per cent. About half-way up the tube is a graduation with the letter U annexed, and still higher up is another with the letter R. The precipitating reagent is prepared by dissolving 10 grms. of picric acid and 20 grms. of citric acid in 900 c.c. of boiling water; when the solution is cold, water is added to 1000 c.c. The tube is filled to the mark U with urine taken from the twenty-four hours' supply; the reagent is then added up to the mark R; the tube is now corked and is gently inverted two or three times so as to mix the liquids without shaking, after which it is placed in the vertical position and is allowed to stand twenty-four hours, when the height to which the precipitate reaches is read off by the graduations. If, after twenty-four hours, the height of the precipitate exceeds the highest of the graduations, the estimation must be repeated with some of the urine that has been diluted with one or more volumes of water, the result obtained being multiplied by two (for equal volumes) or more according to the degree of dilution. Occasionally, after the usual period of standing, the precipitated albumin remains floating in the liquid; in this case the tube should be well shaken and once more left to deposit. When much globulin or compound proteid is present a satisfactory deposition is not attainable. This method is fairly adequate for clinical purposes, inasmuch as a relative estimation of the daily amounts of albumin contained in the urine from the same patient rather than an exact determination is usually what is required. The readings above 1, and below 4 or 5, are the most accurate; the extremes in both directions are unreliable. Tubes which are graduated up to 12 and 14 divisions are quite unreliable, as slight variations in the density of the precipitate throw the readings out of all proportion to the amount of albumin present. The periods allowed for deposition of the precipitate should always be exactly twenty four hours.



FIG. 4.
Esbach's tube.

SERUM GLOBULIN. (Paraglobulin.)

Globulin is insoluble in water. On this fact, W. Roberts founded the simple test of dropping urine into a tall glass jar filled with distilled water; if much globulin is present, each drop produces a milky trail as it sinks in the water, and, after a considerable number of drops have been added, the whole of the water becomes opalescent. If a little acetic acid or liquor potassæ is added to the opalescent water the globulin is dissolved and the water becomes clear.

To a little urine in a test-tube add a drop or two of liquor potassæ so as to render the urine faintly alkaline; then incline the tube and pour down the side some saturated solution of magnesium sulphate, when the presence of globulin will be indicated by the formation of a white precipitate where the two liquids come in contact. (Paton.)

When the salts contained in urine are separated from it by dialysis, the globulin that is present, and that was held in solution by the salts, is precipitated, the albumin remaining in solution.

If urine which contains much globulin is diluted with twice its volume of distilled water, and a drop or two of acetic acid is added, a precipitate is produced which is easily soluble in excess of the reagent. The compound proteids are also precipitated by the same means, but the precipitate is dissolved with difficulty, if at all, in excess of acetic acid.

Serum globulin may be separated from serum albumin by faintly alkalisising the urine and then saturating it with magnesium sulphate; the globulin is precipitated whilst the albumin remains in solution. The same result is attained by half saturating the urine with ammonium sulphate; this may be done by adding to the urine an equal volume of a saturated solution of that salt. When ammonium sulphate is present in solution over 24 per cent. globulin is precipitated; above 33.5 per cent. precipitates some of the serum albumin, all of which is thrown down by ammonium sulphate in saturated solution. A half-saturated solution is equal to 26 per cent. (Kauder.¹)

For clinical purposes an estimation of the relative amounts of albumin and globulin present in urine may be made by Noel Paton's ² method. First, the total proteids are estimated by Esbach's process, then 50 c.c. of the urine is faintly alkalisised and afterwards saturated with magnesium sulphate. After standing twenty-four hours the liquid is filtered, and a measured portion of the filtrate is also dealt with by Esbach's process; this gives the amount of albumin in the urine, allowance being made for the increase in volume caused by the

¹ *Arch. f. exp. Path.*, 1886.

² *Brit. Med. Journ.*, 1890.

presence of the magnesium sulphate. If this is subtracted from the total proteids the difference represents the amount of globulin. By this procedure any compound proteid and hetero-albumose that are present in the urine are reckoned as globulin.

The amount of globulin present in 100 c.c. of urine may be determined by faintly alkalising, and then saturating with magnesium sulphate. The precipitate is collected on a filter, and after being well washed with a saturated solution of magnesium sulphate is dissolved in a very weak saline solution; a few drops of acetic acid are then added, and the solution is boiled so as to coagulate the globulin. The coagulated globulin, collected on a tared filter, is dried at about 110° C., and weighed.

FIBRIN.

Before being tested, fibrin clots should be well washed with a 5 per cent. solution of common salt in order to remove any globulin; the washing is continued until the wash-water ceases to respond to the tests for proteids. Fibrin may then be recognised by its reaction to Weigert's stain, to the xanthoproteic test, and to Millon's reagent. When some of the casts are dissolved by boiling them in half per cent. hydrochloric acid the solution reacts to the tests for albumin.

COMPOUND PROTEIDS.

When nitric acid is allowed to flow gently down the side of an inclined test-tube two-thirds full of urine which contains a compound proteid, a cloud gradually develops about an inch above the stratum of acid which lies at the bottom of the tube. The urine that intervenes between the cloud and the acid remains quite clear, and, on heating, the cloud may, but does not invariably disappear. If the opposite method be adopted—the urine being added to the acid—the compound proteid appears as a sharply defined disc, separated only a short distance from the acid by a layer of clear urine.

To another specimen of the same urine, which must be perfectly limpid, the addition of a few drops of acetic acid (B.P.) develops a turbidity which is dissipated by strong hydrochloric acid, and sometimes by heat; less frequently it is dissipated by excess of acetic acid, which is in favour of the proteid that yields this reaction, being nearly allied to globulin.

If, after the addition of acetic acid, the turbidity of the urine which results is not increased by the subsequent addition of a 10 per cent. solution of potassium ferrocyanide, the presence of a compound proteid is indicated, as opposed to hetero-albumose.

As the salts that are present in the urine keep the compound proteids in solution, it is often necessary to dilute the urine with one or two volumes of distilled water before adding the acetic acid. A still better way is to remove a portion of the salts by dialysis, when a single drop of weak acetic or hydrochloric acid will throw down the compound proteids in a gelatinous form; if the dialysis is carried beyond the stage indicated, and most of the salts are removed, precipitation occurs spontaneously. The most convenient way of dialysing urine is to use a parchment-paper tube; a length of the tube, partially filled with the urine, is suspended by the two ends; the middle and dependent portion which contains the urine is submerged in a large volume of distilled water, which should be renewed two or more times. Six or eight hours dialysis is usually sufficient to render the urine very susceptible to the action of acetic acid.

On applying the boiling-test to urine which contains a compound proteid, and subsequently adding a drop or two of acetic acid, the turbidity produced is usually dissolved by hydrochloric acid; if the turbidity is due to albumin it persists.

The urinary mucin, or mucoid substance, can only be distinguished from the other compound proteids by warming it on the water-bath with dilute hydrochloric acid and then adding an alkaline solution of cupric oxide, which is gradually reduced to the cuprous state. The reducing power thus shown may be compared with that of a specimen of the same urine without previous treatment with hydrochloric acid and heat: any reducing power possessed by the urine is increased by the reducing substance formed on warming with the acid. This, however, fails to distinguish between mucin and chondro-albumin; the chondrosin which is split off from that substance also reduces cupric oxide when heated with it. It would be necessary, therefore, to test a portion of the urine, after it has been heated with the hydrochloric acid, for sulphuric acid, which, if the proteid substance is chondro-albumin, will be split off from the chondroitin-sulphuric acid; any sulphur liberated from mucin would not appear in the oxidised form.

Saturation with sodium chloride does not precipitate urinary mucoid; the other compound proteids are, more or less, thus precipitated.

Nucleo-histon may be distinguished from the rest of the group of compound proteids by not being precipitated on saturation with magnesium sulphate.

It will be seen that in many respects the reactions of globulin, of the compound proteids and of hetero-albumose are very similar; in

some instances their differentiation is only to be achieved by a careful application of several tests, some of which cannot be effectively performed with small quantities of urine. In ordinary clinical work, therefore, it is useless to attempt to determine whether nucleo-albumin, chondro-albumin, or mucin is the compound proteid, the presence of which in the urine is revealed by the tests above described. It will be observed that in the description of the tests it is stated that the turbidity produced by acetic acid may disappear on heating the urine, or by adding an excess of the acid. In dealing with simple solutions of mucin the precipitate caused by acetic acid is not soluble in excess; whereas, under the same conditions, nucleo- and chondro-albumins are soluble in excess, but with difficulty. It would be very unsafe, however, to apply these reactions to a complex solution, like urine, and to read the results rigidly according to this formula. In addition to the extraneous influences brought to bear by the saline constituents, the urea, and extractives of the urine, the difficulty is increased by the composition of the proteids in question being by no means a fixed quantity, and consequently their behaviour to precipitants and solvents is almost certain to vary. The difference in the reaction of the precipitated compound proteids of urine to heat is even more sharply defined: some readily dissolve when the urine is gently warmed, others are permanent at all temperatures up to the boiling-point. Here, also, the same influences, extrinsic and intrinsic, come into play and prevent trustworthy inferences being drawn. For example, the proteid usually present in icteric urines, which formerly was called "biliary mucin," and more recently has been held to consist chiefly of a nucleinic or a taurocholic combination of albumin, or again, as being nearly related to globulin (Staehelin¹), is precipitated by acetic acid in the cold and, usually, is readily dissipated by heat. Occasionally, however, the turbidity does not disappear on warming, and this without any indications of the presence of serum albumin. It is obvious that there is a difference either in the proteid or in the urine, most probably in the former; but it would be generalising on slender premisses to pronounce the first to be taurochol-albumin, and the second to be mucin, on the strength of this one reaction. Again, the precipitate produced by the action of acetic acid in the cold on the mucinoid substance, formerly called "urinary mucin," and now considered to be nucleo- or chondro-albumin, probably associated with urinary mucoid, or, according to another view, fibrino-globulin, is, oftener than not, uninfluenced by heat; sometimes the opalescence due to it is diminished but is not removed. These ambiguous reactions, probably

¹ *Münchener med. Wochenschr.*, 1902.

due to a blend of proteids, render differentiation difficult to the clinical observer; with the relatively small quantities of urine at his disposal and the inadequacy of the tests, the inferences he can draw can be little more than conjectural. Fortunately, however, it is infinitely less important to distinguish between the different members of the compound proteid group than it is to distinguish them as a class from serum albumin: the real question is—does the reaction indicate serum albumin or does it not? With the nitric-acid test the distinction between the mucinoid group and serum albumin is usually not difficult: the precipitate of coagulated albumin commences immediately on the surface of the acid; that due to a compound proteid is higher up the column of urine and is separated from the acid by a stratum of clear urine. Sometimes, when both are present, the albumin deposit, although not very dense, extends a considerable distance up the column of urine, almost, or possibly quite, reaching the position occupied by the mucinoid substance; even then, the albumin cloud fades off as it approaches the mucinoid deposit, which can still be recognised as a distinct zone. This reaction, alone, would render nitric acid an indispensable reagent in urinary-proteid testing. The more sensitive reagents, almost without exception, fail to distinguish between serum albumin on the one hand and compound proteids on the other—both are precipitated immediately over the reagent. It is true that on gently warming the urine the precipitates due to the compound proteids are dissolved, whereas the precipitate due to albumin is not. As an ordinary clinical procedure, however, this is not easily accomplished: a fine disc of opalescence due to albumin may be diffused by the upward movement of the heated urine and vanish without being dissolved, even if the precaution be adopted of dipping the test-tube in hot water instead of holding it over the flame.

ALBUMOSES AND PEPTONE.

If urine is acidulated with acetic acid, and then some 10 per cent. solution of potassium ferrocyanide is added, any primary albumoses that are present are precipitated; on warming, the precipitate is dissolved and it reappears on cooling. The precipitate thus produced—not by the acetic acid but by the subsequent addition of potassium ferrocyanide—distinguishes albumose from the compound proteids; the solution of the precipitate by heat distinguishes it from that due to serum albumin.

The primary albumoses—hetero-albumose for example—when in faintly alkaline solution are precipitated by heat, the precipitate being dissolved by dilute hydrochloric acid. Nitric acid also pre-

precipitates them in the cold; the precipitate dissolves on heating and reappears on cooling. Like globulin, hetero-albumose is precipitated, but less completely, by saturation of the urine with magnesium sulphate. Deutero-albumose, being a later product, more nearly approaches peptone, for which it is often mistaken when present in urine. When urine is saturated with ammonium sulphate at a temperature of 70° C., and is afterwards cooled and filtered, and, after alkalisisation with ammonium carbonate, is again saturated with ammonium sulphate and filtered, the filtrate being made feebly acid with acetic acid and for the third time saturated with ammonium sulphate, and, after being boiled, is once more filtered, all deutero-albumose that may have been present is removed, and, of proteids, the filtrate can only contain peptone. Neither nitric acid nor heat precipitates peptone; but it is precipitated by alcohol, tannin, potassic-mercuric iodide, and partially by phosphomolybdic and phosphotungstic acids. The true proteolytic peptone, however, is not met with in urine.

The Biuret Reaction.—If an equal volume of liquor potassæ, or of a solution of soda, be mixed with some urine and then a couple of drops of a very dilute solution of copper sulphate be added, a rose-red colour is produced both with albumoses and with peptone; with the same reagents albumin gives a violet colour. In order to demonstrate the presence of peptone all other proteids must be removed from the urine by precipitation with ammonium sulphate, as described above; if the filtrate yields the biuret reaction, it has been accepted as evidence that it contains peptone. Stokvis¹ and Salkowski² have pointed out that urine which contains urobilin often gives the biuret reaction. Salkowski considers that the risk of mistaking this reaction for that due to albumoses will be obviated by spectroscopic examination of the urine. If the urobilin band is present the biuret reaction cannot be accepted as evidence of the presence of albumose or peptone; for although lead acetate removes urobilin from the urine, it is not applicable when testing for albumoses inasmuch as it carries down some albumose along with the urobilin, and consequently, if only a small amount of albumose be present, it may in this way escape detection. Stokvis is inclined to doubt the accuracy of many of the reported cases of peptonuria, and attributes the biuret reaction obtained in these cases to urobilin; moreover, he states that lead acetate does not precipitate all the urobilin, and that phosphotungstic acid and other reagents which precipitate urobilin also precipitate albumoses.

¹ *Zeitschr. f. Biol.*, 1898.

² *Berliner klin. Wochenschr.*, 1897.

Histon may thus be detected (Jolles¹). To 50 c.c. of the urine, free from albumin, sufficient 4 per cent. acetic acid is added to make the reaction slightly acid, and then 10 per cent. solution of barium chloride, with constant stirring, as long as a precipitate forms. After standing half an hour the clear liquid is decanted and the precipitate poured on a filter, and, without washing, is placed along with the filter in a beaker with 10 c.c. of 1 per cent. hydrochloric acid and allowed to stand three or four hours. Sodium carbonate is added to alkaline reaction in order to precipitate the barium chloride, and the solution is filtered and divided into two parts: one is tested for the biuret reaction, and to the other, after carefully acidulating with hydrochloric acid, a little ammonia is added; the presence of histon is indicated by a distinct turbidity.

The **Bence Jones proteid** gives the following reactions: When heated to from 58° to 65° C. the proteid coagulates in a gelatinous form; when heat is very slowly applied, a slight turbidity may appear at about 50° C. At 70° C. to 80° C. the urine begins to be less turbid and at 100° C. the coagulum is mostly or entirely dissolved. Nitric acid produces a dense precipitate which disappears on warming and returns on cooling. Hydrochloric acid acts in the same way. Acetic acid (30 per cent.) produces no precipitate (Magnus-Levy); when nearly half volume of 50 per cent. acetic acid is used a gelatinous condition ensues in three or four minutes, so that the test-tube can be inverted without escape of its contents; on warming, the gelatinous mass liquefies (Grutterink and Graaf²). Carbon dioxide passed through the urine diluted with ten volumes of water gives no precipitate. Picric acid, and tannin with acetic acid, give copious precipitates which are only slightly soluble on boiling. Ferrocyanide of potassium with acetic acid produces a scanty precipitate which is partially dissolved on boiling and returns on cooling. Acetic acid and saturated sodium chloride solution completely precipitate the proteid, as also does a double volume of a saturated solution of ammonium sulphate; whereas saturation with magnesium sulphate has no effect. Salicyl-sulphonic acid produces a copious precipitate which dissolves on heating and reappears on cooling. The biuret-test gives a reddish-violet colour. The proteid will not dialyse.

¹ *Zeitschr. f. physiol. Chem.*, 1898.

Ibid., 1901.

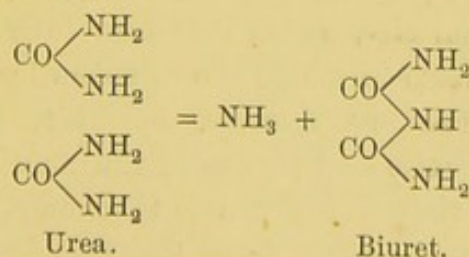
NITROGENOUS SUBSTANCES.

ALMOST all the nitrogen that is eliminated from the body is excreted by the kidneys; the amount varies from about 12 to 15 grms. in the twenty-four hours, so that in the average daily quantity of urine the percentage present is from 0.8 to 1.05. The largest portion of the nitrogen, about 84 to 90 per cent., appears in the urine as urea, about 4 per cent. as ammonia, 3 per cent. as creatinin, 2 per cent. as uric acid, along with the other purins; the remainder is represented by hippuric acid, the chromogens and pigments, with traces of other nitrogenous substances. About 7 or 8 per cent. of the output of nitrogen is excreted in the fæces. The urinary nitrogen is increased by copious draughts of water and by all conditions which further the assimilation of nitrogenous foods, the balance being withdrawn from the fæces. Soon after meals an increase in the excretion of nitrogen sets in, and reaches its maximum in five or six hours.

During the early days of infantile life, 7 to 8 per cent. of the total nitrogen is excreted in the urine as uric acid. This sinks to less than one-half at the time of the formation of the uric acid-infarcts in the kidneys (Sjöqvist ¹).

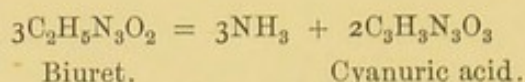
UREA. $\text{CO}(\text{NH}_2)_2$.

Urea, or carbamide, is a diamide of carbonic acid. It is a white substance which crystallises in rhombic prisms. It is soluble in its own weight of cold water and in five parts of alcohol. It has a neutral reaction, although it possesses basic properties and combines with one equivalent of acids to form salts. It melts at 130°C . At 150°C . it gives off one molecule of ammonia from two molecules of urea, biuret being formed:

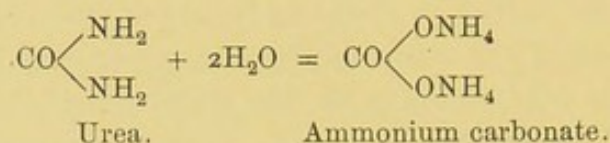


¹ *Nordisk. med. Arkiv*, 1894.

When biuret is dissolved in water and is alkalised with potash, a drop of a weak solution of copper sulphate produces a pink colour—the biuret reaction. Above 170° C. biuret is resolved into ammonia and cyanuric acid.



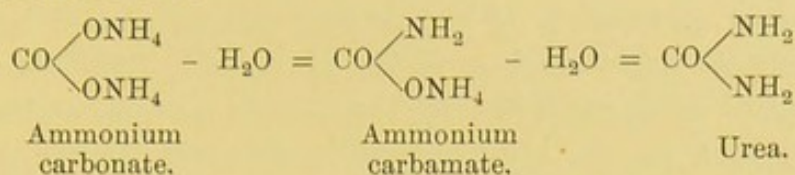
When urea is heated with a strong solution of potash or soda hydrolysis occurs with the liberation of CO_2 and NH_3 , the ammonia being partly evolved. This decomposition is also effected by certain micro-organisms, especially the *Micrococcus ureæ*, with the formation of ammonium carbonate, constituting the alkaline fermentation of urea :



Urea forms crystalline combinations with several acids, of which the nitrate $\text{CO}(\text{NH}_2)_2\text{HNO}_3$, and the oxalate $\text{CO}(\text{NH}_2)_2\text{H}_2\text{C}_2\text{O}_4$, are the most important. The nitrate is formed by adding nitric acid in excess to a strong solution of urea. Crystals of urea nitrate, which take the form of colourless, thin rhombic tables, are deposited; they are easily soluble in water, but are much less so in water which contains free nitric acid. The ease with which these crystals are formed may be taken advantage of as a test for urea. If a drop of urine that contains much urea is placed on a microscope slide and is covered with a thin cover-glass, under which a drop of nitric acid is allowed to run so as to mix with the urine, the formation of crystals of urea nitrate may be observed with the microscope. Urea oxalate is formed by adding a saturated solution of oxalic acid to a strong solution of urea; the crystals formed resemble those of the nitrate, but they are less soluble in water.

Urea, the most prominent physiological constituent of urine, is the chief representative of the ultimate product of proteid metabolism. The amount of proteid decomposition may be estimated by multiplying, by the factor 6.3, the daily weight of nitrogen excreted; the product gives the proteid equivalent. Proteid metabolism mostly takes place in the muscles, which therefore furnish the nitrogen of urea. The actual form in which the nitrogen leaves the muscles is undetermined; there are reasons, however, for believing that ammonia and sarcolactic acid represent the final products of muscle metabolism. It is therefore probable that ammonium lactate is the form in which nitrogen parts from the muscles. Combinations of organic acids with ammonia are converted by the tissues into

carbonates, which undergo dehydration in the liver-cells, so that ammonium carbonate, losing one molecule of water, is converted into ammonium carbamate, which by withdrawal of another molecule of water becomes urea :



Zillessen,¹ by tying the hepatic artery in rabbits, determined the occurrence of ammonium lactate in the urine; and Marfori² found that when ammonium lactate is injected into the veins of a dog it is excreted as urea. The results recently obtained by Ascoli,³ however, tend to show that the path followed by the products of proteid metabolism through ammonium lactate and carbamate to urea is not the only one; other sequences occur through many different intermediate products, amongst which are the hexon bases—that is, cleavage products of protamine, the simplest form of the proteid substance.

Although most of the urea is formed in the liver, it appears possible that some may be formed elsewhere; for whilst extirpation or other method of abolishing the function of the liver is undoubtedly followed by material diminution in the formation of urea, it is not entirely arrested. In some cases of profound disturbance of the liver function, in advanced hypertrophic biliary cirrhosis and in cancer of the liver, both the absolute and the relative amounts of urea and ammonia have been found not to be materially altered (Münzer⁴).

Urea is present in urine at the rate of from 2 to 3 per cent., in the blood about 0.03 to 0.1 per cent., in the sweat from 0.05 to 0.01 per cent., and in other secretions and tissues. It is probably not present in the muscles, although, as just stated, they furnish the nitrogen from which the urea is formed. Schöndorff,⁵ however, found over 0.1 per cent. and Blaikie⁶ 0.02 per cent. of urea in the muscles of dogs.

A healthy adult man fed on ordinary mixed diet excretes a fairly constant amount, 30 to 35 grms. (500 grains) of urea in the twenty-four hours women excrete rather less. In proportion to the body-weight children excrete more urea than adults: a man excretes 0.4 to 0.6 grm. per kilo of body-weight, whilst a child excretes nearly 1 grm. per kilo; the absolute amount excreted

¹ *Zeitschr. f. physiol. Chem.*, 1891.

² *Gazz. d. Ospedali*, 1901.

³ *Pflüger's Arch.*, 1900.

⁴ *Arch. f. exp. Pathol.*, 1893.

⁵ *Arch. f. exp. Pathol.*, 1894.

⁶ *Journ. of Physiol.*, 1898.

by the child is obviously less than that of the adult. If the daily amount of urea is divided by 2, an approximate estimation of the total nitrogen-excretion is obtained. The conditions which promote the excretion of urea are: large amounts of nitrogenous food and excessive metabolism of the proteid tissues, caused by severe bodily exercise or by various kinds of disease. An estimate of the amount of urea furnished by the tissues on the one hand, and directly by food on the other, may be arrived at, as suggested by Salkowski,¹ by determining the relation borne by the sodium chloride to the urea. In the healthy state, and under normal conditions as to food, the daily excretion of urea is about double that of sodium chloride. On the other hand, in wasting diseases in which but little food is eaten, the urea, being mostly derived from tissue-metabolism, proportionally exceeds this ratio. In diabetes, however, the sodium chloride introduced by the excessive amount of animal food that is eaten maintains the ratio notwithstanding the coincident rapid tissue metabolism.

The excretion of urea is increased in the active stage of fevers, in acute inflammatory diseases, in acute wasting diseases due to various causes, and in the first few days after childbirth; in diabetes it may exceed three or four times the normal amount. It is diminished in chronic diseases, and whenever the intake of nitrogenous food is greatly reduced, as in starvation; after the crisis of acute febrile diseases it is usually lessened for a time. In some pathological conditions the daily excretion of urea is diminished, although the total nitrogen of the urine is but little altered. In acute atrophy of the liver, in acute phosphorus poisoning, and in the early stage of cirrhosis, the urea may represent only half the total nitrogen (in the first two it may be reduced very much lower), the rest being present chiefly in the form of ammonia and other intermediate products of nitrogenous decomposition. In some diseases, v. Jaksch² found that an increased amount of nitrogen is excreted in products like the amido acids (including hippuric acid, allantoin, oxypoteic acid and other unnamed analogous bodies) at the expense of the urea. In health, from 1.5 to 3 per cent. of the total nitrogen consists of amido acid-nitrogen, the amount being increased by the ingestion of benzoic acid-containing substances. In typhoid fever, in diseases of the liver, and in diabetes insipidus, an increased amount of nitrogen is excreted in products like the amido acids; in one case of diabetes insipidus the amido acid-nitrogen amounted to 49.40 per cent., whilst the urea nitrogen only reached 47.70 per cent. In a healthy man, when

¹ *Die Lehre v. Harn*, 1882.

² *Zeitschr. f. klin. Med.*, 1902 and 1903.

food is abruptly cut off, the excretion of urea suddenly drops, and, for a few days, remains fairly constant, with a tendency to still further diminish which is progressive as long as food is withheld; the same holds good in respect to the total nitrogen of the urine. Paton and Stockman¹ give the following average daily amounts of urinary nitrogen for six consecutive periods of five days, during thirty days fasting: (1) 11.9 grms., (2) 5.4 grms., (3) 5.1 grms., (4) 4.2 grms., (5) 4.2 grms., (6) 3.1 grms.

Occasionally urea is excreted in abnormal amounts by other organs than the kidneys. It has been found in large amount in the expectoration from cases of bronchitis and pneumonia; the skin also has been known to excrete urea so abundantly as to present the appearance of having been dusted over with a white crystalline powder. Jahnel² relates a case of chronic nephritis in which this condition occurred during the last stage of the disease. Urea may be present in entirely abnormal situations; in the contents of the stomach, for example.

The *presence of urea* in dilute solution may be demonstrated by the addition of a little solution of mercuric nitrate; a flocculent precipitate is produced which is soluble in a solution of sodium chloride.

If a few drops of a concentrated solution of furfurol are added to a crystal of urea, and then a drop or two of 10 per cent. hydrochloric acid, a yellow colour is at once produced which changes through green, blue, and violet into purple-red (Schiff).

QUANTITATIVE ESTIMATION OF UREA.

The method usually adopted is to decompose the urea of a measured quantity of urine by means of a solution of sodium hypobromite (or of sodium hypochlorite) containing a large excess of sodium hydrate. The products of decomposition are carbon dioxide and nitrogen: $\text{CO}(\text{NH}_2)_2 + 3\text{NaBrO} = 3\text{NaBr} + 2\text{H}_2\text{O} + \text{CO}_2 + \text{N}_2$; the former is absorbed by the free alkali, and the volume of nitrogen, of which 10 c.c. in the moist state and at ordinary temperature and pressure equals .25 gm. urea, is measured in a tube which is so graduated that each division represents 0.1 per cent. of urea. A convenient form of apparatus is represented by Fig. 5. It consists of a measuring-tube graduated so as to show the percentage of urea; by rubber tubing this is connected at its lower end with a levelling-bulb, which can be fixed at any height by means of a support and thumb-screw. The upper end of the measuring-tube is connected with a generating flask.

¹ *Proc. Roy. Soc. Ed.*, 1889.

² *Wiener med. Presse*, 1897.

The hypobromite solution is prepared by mixing 2.5 c.c. of bromine with 25 c.c. of a solution of sodium hydrate made by dissolving 100 grms. in 250 c.c. of water. As the hypobromite solution tends to deteriorate by keeping, it should be

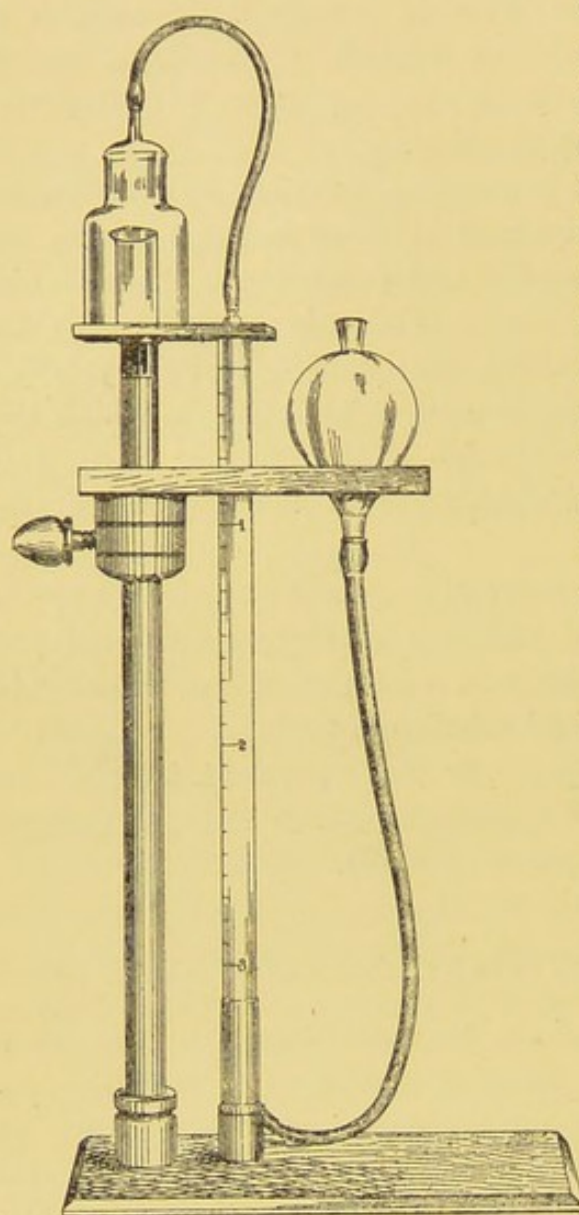


FIG. 5. Ureameter (the author's form).

freshly made for each estimation of urea. The ureameter is prepared by removing the stopper of the flask, raising the levelling-bulb to its highest point, and then pouring in water until the measuring-tube is filled. The solution of hypobromite, when cold, is poured into the inner receptacle of the generating flask and 5 c.c. of the urine to be examined are delivered from a graduated pipette into the outer compartment of the flask. The flask, after being closed with the stopper, is then inclined so as to allow the hypobromite solution gradually to mix with the urine, the flask being gently agitated so as to promote the decomposition of the urea. As the nitrogen drives down the column of water in the measuring-tube, the bulb is lowered so that the level of the water in it is at the same height as that of the water in the tube. After the

whole of the hypobromite has been added to the urine the apparatus is allowed to stand for at least five minutes when the final adjustment of the water-levels is made, and the volume of nitrogen is read off in percentages of urea.

If albumin is present a couple of drops of acetic acid should be added to a little of the urine, which should then be boiled, cooled, and filtered before its urea is estimated. If more than 3 per cent. of urea be present, 2.5 c.c. of the urine, diluted with an equal volume of water, should be used in place of 5 c.c. of urine; if this be done the yield of nitrogen must be multiplied by two.

For clinical use the hypobromite process is the most convenient, and it is sufficiently accurate; although under ordinary conditions only about 93 per cent. of the urea-nitrogen is liberated by it. Any additional nitrogen afforded by the decomposition of the hippuric acid, creatinin, and the purin bodies, is not enough to make good the deficiency. As, however, the evolved nitrogen is usually measured in the moist state and without correction for pressure, and possibly at a slightly higher temperature than 60° F., the deficiency is sufficiently compensated.

The presence of sugar in the urine increases the evolution of nitrogen from the urea up to 99 per cent. of the theoretical amount; in consequence of this, Allen¹ recommends that the percentages obtained from diabetic urine should be multiplied by 0.93 in order to equalise the results with those obtained from urine which does not contain sugar.

The number of grains of urea in each fluid ounce of urine is obtained by multiplying the percentage by the factor 4.375.

When an exact determination of the amount of urea in urine is required the other nitrogenous bodies must be removed, and then the nitrogen of the isolated urea must be accurately determined. This is best done by the Mörner-Sjöqvist² process, which consists in adding a mixture of barium chloride and hydrate to the urine along with ether and alcohol; all the nitrogenous constituents, except the urea, are precipitated. The urea is held in solution by the ether-alcohol and its nitrogen is determined by Kjeldahl's process. This method may be conveniently carried out as slightly modified by Bödtker.³

A solution is prepared consisting of 50 grms. of barium hydrate and 350 grms. of barium chloride to the litre; of this 2.5 c.c. are poured into a stoppered flask along with 2.5 c.c. of the urine and 75 c.c. of a mixture of one volume of ether with two volumes of 90 per cent. alcohol. After being well shaken the flask is allowed to stand for twenty-four hours, when the contents are filtered into a porcelain dish, the precipitate being washed with more ether-alcohol which is added to the filtrate. At a temperature between 50° and 60° C. the filtrate, with the addition of half a gramme of magnesia, is slowly evaporated down to 20 c.c.; this rids it of any ammonia. The concentrated filtrate is then submitted to Kjeldahl's process: 10 c.c. of strong sulphuric acid are cautiously added and the dish is placed on a water-bath, which is kept boiling until no further loss occurs from evaporation; the contents of the

¹ *Chemistry of the Urine*, 1895.

² *Skandin. Archiv*, 1891.

³ *Zeitschr. f. physiol. Chem.*, 1893.

dish are then emptied into a flask, any adhering matter being washed into the flask with distilled water. The flask, placed on wire netting, is heated over a Bunsen flame for two hours, when the urea will have been split up into carbon dioxide and ammonia; the former is evolved, and the ammonia remains in solution combined with the acid. Excess of sodium hydrate is then added, and the free ammonia is distilled into a measured quantity of decinormal sulphuric acid, the balance of free acid being subsequently ascertained by titration. The percentage of nitrogen multiplied by 2.14 gives the percentage of urea.

Folin¹ splits up urea by subjecting it to the high temperature at which magnesium chloride boils. Crystallised magnesium chloride melts at 115° C. and the liquid thus produced boils at 160° C. In a flask furnished with a reflux-condenser, 3 c.c. of urine, 20 grms. of magnesium chloride and 2 c.c. of hydrochloric acid (1.14) are boiled. After the excess of water is boiled off, the contents of the flask are kept boiling for 45 minutes; they are then diluted with water, and transferred to a flask of one litre capacity; 7 c.c. of 20 per cent. solution of soda are added, and the ammonia which has been split off is distilled (the distillation being kept up for one hour), and titrated in the usual way. Uric and hippuric acids yield no ammonia by this process. Mörner² finds, when the urine is first treated by the Mörner-Sjöqvist process, that the method gives more accurate results than the other methods in use. Applied direct to the urine, it also gives good results, but the presence of allantoin may occasion error. The direct process is not applicable to urine which contains sugar; but the estimation of urea in saccharine urine may be made after treatment by the Mörner-Sjöqvist process.

Another method, modified by Pflüger and Bleibtreu,³ consists in precipitating, by means of phosphotungstic and hydrochloric acids, all nitrogenous bodies, except urea, which is then decomposed by heating it to 250° C. for about three hours with crystallised phosphoric acid. The ammonia thus formed is estimated, as in the process previously described.

It is stated by Salaskin and Zaleski⁴ that the ether-alcohol of the first described process takes up hippuric acid as well as urea. Braunstein,⁵ who corroborates this, recommends that the residue after evaporation of the ether-alcohol extract should be decomposed by phosphoric acid at a temperature not exceeding 145°, by which the nitrogen of the urea only and not that of the hippuric acid will

¹ *Zeitschr. f. physiol. Chem.*, 1900 and 1902.

² *Skandin. Arch. f. Physiol.*, 1903.

⁴ *Zeitschr. f. physiol. Chem.*, 1899.

³ Pflüger's *Archiv.*, 1889.

⁵ *Ibid.*, 1900.

be set free. Mörner and Sjöqvist¹ state that phosphotungstic acid carries down some of the urea, along with the other nitrogenous constituents, and therefore that this process gives too low a result.

When a determination of the total amount of nitrogen in urine is required, 2.5 c.c. of the urine are directly subjected to Kjeldahl's process, as just described, the preliminary treatment for removing other nitrogenous constituents than urea being omitted.

ALLOXUR or PURIN BODIES.

The alloxur bodies comprise those substances into the constitution of which an alloxan and a urea nucleus enter, the most important member of the group being uric acid.

Fischer gives the name *purin bodies* to these substances as they include in their composition the purin nucleus C_5N_4 .

URIC ACID. $C_5H_4N_4O_3$.

Uric acid is a white crystalline powder composed of small rhombic prisms or tables, which are devoid of taste and odour. Uric acid is very slightly soluble in cold water, the solubility being usually stated as 1 : 15,000 or 16,000. His and Paul² have re-determined the ratio with pure water at 18° C., and give it as 1 : 39,480; they find that a litre of water when saturated with uric acid only dissolves 0.0253 grm. Uric acid is more soluble in boiling-water, 1 : 1800. It is insoluble in alcohol and ether. It is soluble in solutions of the fixed alkalies and many of their salts—the acetates, phosphates, carbonates, and lactates; it is but slightly soluble in ammonia and its salts. It is dissolved by sulphuric acid from which, on the addition of water, it is precipitated unchanged. When strongly heated, uric acid is decomposed into urea, cyanuric acid, hydrocyanic acid, and ammonia. By the action of an oxidising agent, such as nitric acid, uric acid yields urea and alloxan. Uric acid possesses the property of reducing the cupric tartrate of Fehling's solution, usually to the red cuprous oxide; its reducing power is not very energetic, and consequently prolonged boiling is needed to bring it into action.

Uric acid is present in urine in combination with bases forming salts which are much more soluble than the free acid. Under certain conditions uric acid is displaced from its combinations and crystallises out of the urine, either in the calices of the kidneys, in the bladder, or after the urine is voided. If crystals of uric acid are deposited by

¹ *Loc. cit.*

² *Zeitschr. f. physiol. Chem.*, 1900.

urine soon after it has been voided, there is reason to fear that some may be deposited in the kidneys, or in the lower urinary passages, and may thus give rise to calculus; the risk of this occurrence is much less if, after the urine is passed, several hours elapse before any crystals appear. After standing a considerable time most urines spontaneously deposit uric acid. Early spontaneous precipitation of uric acid does not necessarily indicate that it is present in the urine in excess. Early deposition has been attributed to a highly acid urine which is poor in salts; but Jerome¹ states, as the result of experimental investigations, that the tendency to deposit uric acid is not always due to high acidity nor to high percentage of uric acid, although such conditions may favour precipitation. In 1892 Roberts² stated that the urinary pigments play an important part in preventing the precipitation of free uric acid in febrile urines. He observed that deeply coloured, sharply acid urines, though they deposit urates, are not prone to deposit free uric acid; and that the absence of pigments in the urine favours the deposition of free uric acid. Ten years subsequently, apparently without knowledge of these observations, Klemperer³ arrives at the same conclusions and states that febrile urine which deposits urates seldom deposits uric acid; whilst diabetic urine, which is almost free from pigment and is very dilute, often deposits uric acid. The explanation he gives is that colloidal substances in urine can hold free uric acid in physical solution, and that urochrome is a colloidal substance which possesses this property in a considerable degree. By shaking urine with pure, finely powdered animal charcoal and filtering, it is deprived of its urochrome, and it then readily deposits uric acid. Klemperer⁴ also states that, in urine which has an acid reaction, the solubility of uric acid is diminished by the presence in the urine of much carbon dioxide. Gotto⁵ found that nucleinic acid has a powerful restraining influence on the precipitation of uric acid from aqueous solutions that are acidulated with hydrochloric acid.

The constitutional formula of uric acid shows the close relationship between it and urea—

$$\text{CO} \begin{array}{c} \nearrow \text{NH} \cdot \text{CO} \cdot \text{C} \cdot \text{NH} \\ \searrow \text{NH} \quad \quad \parallel \\ \quad \quad \quad \text{C} \cdot \text{NH} \end{array} \text{CO.} \quad \text{This is further}$$

shown by the fact that in nearly all the decompositions of uric acid a molecule of urea is produced. An aqueous solution of uric acid which had been kept for a year was found by Gigli⁶ to have been spontaneously converted into urea.

¹ *Journ of Physiol.*, 1898.

² *Croonian Lecture*, 1892.

³ *Congress f. inn. Med.*, 1902.

⁴ *Zeitschr. f. diätet. u. physikal. Therap.*, 1901.

⁵ *Zeitschr. f. physiol. Chem.*, 1900. ⁶ *Chemikerztg.*, 1901.

Horbaczewski synthetically produced uric acid by heating together urea and glycocine; this led to the supposition that it may be similarly formed in the system by conjugation of urea with a substance of the nature of an amido acid, such as glycocine; by some, the conjugation is supposed to take place in the liver (v. Schröder, Minkowski¹); by others in the kidneys (A. Garrod, Luff²). Wiener³ finds, in dogs, that the liver is able to some extent to conjugate urea with certain non-nitrogenous substances, such as glycerol, and thus to produce uric acid. The view commonly held at the present time is that uric acid is a product of the cleavage of nucleins. Kossel⁴ proved that the xanthin bases can be obtained from nuclein, and in 1882 surmised that hypoxanthin would probably be found to be an antecedent of uric acid. Horbaczewski⁵ has shown that the alloxur bases can be obtained from the splenic pulp, and that the nuclein it contains will yield uric acid; on this and other grounds he founded the theory that uric acid is derived from disintegration of the leucocytes, and that hyperleucocytosis tends to its production. So far back as 1858 Ranke⁶ demonstrated in certain cases of leucocythæmia that the excretion of uric acid is increased. It is probable that uric acid is derived from two sources: one portion—the endogenous—is supposed to be formed in the course of tissue metabolism from the systemic nuclein-containing substances; the other portion—the exogenous—is derived from the purin or alloxur bases of food.

Horbaczewski's view of the origin of uric acid has been largely accepted; but there are grounds for doubting its universal application, especially as to the rôle played by hyperleucocytosis in the production of the endogenous moiety. Whilst it is true that great excess of uric acid has been found in cases of leucocythæmia, the converse does not invariably hold good. In the case of a woman suffering from Malta fever, in whom the leucocytes ranged from 1500 to 3000 in the cubic millimetre, Hutchison and McLeod⁷ found that on purin-free diet the excretion of the alloxur bases showed no distinct deviation from the normal. In a case of enterica with hypoleucocytosis, Pope⁸ found the excretion of uric acid and of xanthin bases to be of average amount, the relation between them being as usual.

¹ *Arch. f. Physiol.*, 1880. *Arch. f. exp. Pathol.*, 1896.

² *Proc. Roy. Soc.*, 1893. *Gout: Pathology and Treatment*, 1898.

³ *Beiträge z. chem. Physiol. u. Pathol.*, 1902.

⁴ *Zeitschr. f. physiol. Chem.*, 1882.

⁵ *Sitzungsber. d. k. Akad. d. Wissensch.*, 1891.

⁶ *Ausscheid. d. Harnsäure*, 1858.

⁷ *Journ. of experim. Med.*, 1901. ⁸ *Centralbl. f. innere Med.*, 1899.

It is to be borne in mind that whilst a diminution in the number of the leucocytes of the blood is capable of being microscopically demonstrated, it by no means follows that they alone yield the uric acid-forming nuclein; it is more than probable that the nuclein-containing tissues throughout the body contribute towards the formation of the endogenous uric acid and alloxur bases.

Our knowledge of the exogenous portion of uric acid is more direct; there is ample evidence to show that it is derived from the purins or alloxur bases of food. Food that is rich in nuclein, such as the thymus gland, greatly increases the output of uric acid; for this reason the extractives obtained from ordinary animal food (not the proteids) cause an increase. Siven¹ found that the daily addition of a litre of broth to a rigorous vegetable diet increased the daily excretion of uric acid from 0.34 to 0.79 grm. All foods, both animal and vegetable, that are rich in purins—such as sweetbread, Liebig's extract, and asparagus, when eaten cause an increase in the excretion of uric acid. In certain foods the purins are largely present as free purins, to the rapid absorption of which the early rise of uric acid after a meal is probably due.

By some, uric acid is regarded in the same light as urea—as an end-product; others regard it as an intermediate product. Burian and Schur² give reasons for believing that, in mammals, both the endogenous and the exogenous uric acid is an intermediate product which undergoes further decomposition in the body; but although the capacity to destroy both endogenous and exogenous uric acid exists in the organism, a fraction circulates unchanged in the blood and is excreted intact. In human beings fully one-half of the uric acid that reaches the circulation is excreted unchanged; the other half is destroyed in the liver and various organs through which the blood carries it, and is excreted either in the lowest metabolic state as urea or as a product, such as allantoin, which is intermediate between uric acid and urea.

A. Garrod,³ v. Jaksch,⁴ Luff,⁵ and others state that, in the normal condition, no uric acid is present in the blood; and chiefly on this statement is founded the view that uric acid is formed in the kidneys. Abeles⁶ and other investigators have found uric acid in very small amount in normal blood. Under pathological conditions, on the other hand, uric acid has been found in the blood in relatively large quantities, in gout, in pneumonia, in septic diseases such as gonorrhoeal rheumatism, in hysteria (Petrén⁷), and in all diseases which

¹ Skandin. Arch. f. Physiol., 1900. ² Arch. f. ges. Physiol., 1901.

³ Lumleian Lects., 1883.

⁴ Deutsche med. Wochenschr., 1890.

⁵ Loc. cit. ⁶ Wiss. med. Jahrb., 1887. ⁷ Arch. f. exp. Pathol., 1898.

are accompanied by leucolysis. In contracting kidney v. Jaksch¹ and Klemperer² always found uric acid in the blood; in chronic lead poisoning Garrod found an excess.

In normal urine the daily amount of uric acid is about 0.3 to 0.7 grm.; or, with an average secretion of urine, from about 0.024 to 0.060 per cent.; if large quantities of animal food, rich in purins, are eaten, the daily amount may be increased to 2 grms. The excretion of uric acid is very constant for each individual; but the amount varies considerably for different individuals (Marés³). After a meal which is composed of food rich in purins, the increase in excretion of uric acid begins in about two hours, and the maximum output takes place from about the second to the fifth hour. It is noteworthy that the increase begins and ends more rapidly than that of the other urinary nitrogenous substances.

In disease an increase in the uric acid excretion occurs in fevers, on account of the excessive proteid metabolism; in profound leucocythæmia as much as 4 grms., and in a unique case, recorded by Magnus-Levy,⁴ 8.72 grms. were excreted in the twenty-four hours. In pernicious anæmia an increase often occurs; in simple anæmia and chlorosis, on the other hand, the excretion may be less than the normal. A. Garrod,⁵ W. Roberts,⁶ Luff,⁷ and others hold that during an attack of acute gout the excretion of uric acid is diminished, so that at such times the urine contains less than the usual amount. On the other hand, Pfeiffer⁸ and Badt⁹ found no diminution, but rather an increase during the acute stage of gout. His¹⁰ states that the average daily excretion of uric acid by gouty persons does not differ from the normal average; but, as is the case with healthy subjects, the excretion is subject to considerable and unaccountable variations. He also states that an attack of acute gout is ushered in by a diminution in the uric-acid excretion, which precedes the attack by from one to three days; after the attack an increase occurs which reaches its maximum in from one to five days. The average daily excretion of uric acid during the attacks of gout, and during the intervals, shows no material difference. Camerer¹¹ also observed no difference in the amount of uric acid in the urine of patients suffering from gout and in the urine of healthy people; but he noticed that uric acid crystallised out soon after the urine

¹ *Deutsche med. Wochenschr.*, 1890.

² *Ibid.*, 1895.

³ *Arch. slaves de Biologie*, 1888.

⁴ *Virchow's Archiv*, 1898.

⁵ *A Treatise on Gout*, 1876.

⁶ *Croonian Lectures*, 1892.

⁷ *Gout: Pathology and Treatment*, 1898.

⁸ *Verhandl. d. Congress. f. innere Med.*, 1888.

⁹ *Zeitschr. f. klin. Med.*, 1899. ¹⁰ *Ibid.*, 1899. ¹¹ *Zeitschr. f. Biol.*, 1890.

was voided, whether it was present in large or in small amount. Waldvogel and Hagenberg¹ state that in diabetes, whether complicated with gout or not, the uric acid and the sugar increase and diminish together, unless coma threatens, when the sugar increases and the uric acid decreases. In a case of rheumatoid arthritis Bain² found that the daily excretion of uric acid was reduced to 0.266 grm. In the early stage of cirrhosis of the liver, an increase in the output of uric acid has been observed with subsequent marked diminution. It has been stated that the inhalation of oxygen diminishes the amount of uric acid in the urine and that a restricted supply of oxygen, as occurs in various lung diseases accompanied by dyspnœa, has a contrary effect; this is denied by Senator, Naunyn, and others.

The influence of drugs on the excretion of uric acid is very uncertain. It was formerly supposed that the administration of alkalies materially aided its excretion, lithium salts being regarded as especially potent solvents and eliminators, but the results of experimental research are opposed to this supposition. His³ found that sodium bicarbonate exercises no material influence, and that lithium carbonate appears to diminish the excretion. Haigh⁴ has shown that the administration of sodium salicylate causes an increase in the amount of the uric acid in the urine, an observation that has been corroborated by Magnus-Levy,⁵ who found that it doubled the output, and by other observers. Bohland,⁶ whilst agreeing that sodium salicylate increases the urinary uric acid, found that it also greatly increases the number of leucocytes in the blood; and as, according to Horbaczewski's theory, hyperleucocytosis increases the output of uric acid, the increased excretion caused by sodium salicylate is the result of over-production and not of the elimination of that which has been retained in the tissues. Schreiber and Zaudy⁷ found, on administering to a man 3 grms. of sodium salicylate daily for five consecutive days, that the amount of uric acid was increased on the first day, but that it fell on the second day and during the remaining three days, when it came down to the same level as before the drug was taken. This they attribute to an acquired indifference of the system to the action of the salicylate, which in the first instance produced leucocytosis. After the administration of 10 grms. of sodium salicylate in three days, Ulrici⁸ found that the uric acid

¹ *Centralbl. f. Stoffwechsel-u. Verdauungskrankh.*, 1900.

² *Edin. Med. Journ.*, 1900.

³ *Zeitschr. f. klin. Med.*, 1900.

⁴ *Uric Acid*, 1896.

⁵ *Zeitschr. f. klin. Med.*, 1889.

⁶ *Münchener med. Wochenschr.*, 1899.

⁷ *Deutsches Arch. f. klin. Med.*, 1899.

⁸ *Arch. f. exp. Pathol.*, 1901.

excretion was increased 50 per cent., but he does not believe that the increase is due to leucocytosis. Magnus-Levy considers that the increased amount of uric acid in the urine (1 to 1.25 grms.) after the administration of sodium salicylate is too great to be accounted for by the theory of hyperleucocytosis; he is disposed to attribute it to diminished oxidation of uric acid, which, under other conditions, would be further dealt with in the organism. Even drugs which have a distinct solvent action on uric acid, *in vitro*, such as piperazin, either diminish its excretion or act negatively (Grawitz¹). Urotropine has a slight solvent action on uric acid *in vitro*, but its effect as an excretory adjunct is very doubtful. Nicolaier² is unable to advance any reliable evidence in its favour, and His³ found that, in gout, it seemed on some occasions to increase and on others to diminish the output of uric acid.

Bohland⁴ and others have found that tannic acid and quinine, in daily doses of from 1 to 3 grms., diminish the excretion of uric acid, the first named so powerfully as to inhibit the usual effects of the ingestion of the nuclein-rich thymus gland. It has been found that the administration of quinic acid and its combinations with urotropine, urea, and other bodies increases the formation of hippuric acid, and it is stated by some that this increase is at the expense of uric acid formation (Weiss,⁵ Lewin⁶). Consequently, in the treatment of gout, drugs of this class have been used to lessen the formation of uric acid. This inhibitory action of quinic acid compounds on the formation of uric acid has been denied by Lavadowski,⁷ Nicolaier,⁸ and others. Hupfer⁹ agrees that the administration of quinic acid increases the formation of hippuric acid, but he does not find that it causes any diminution in the output of uric acid.

The moderate use of alcohol either produces no effect on the excretion of uric acid, or tends slightly to increase it; in excess, alcohol may profoundly depress the excretion.

The attempt has been made to establish a normal ratio between the daily excretion of urea and of uric acid; the variations, however, are too wide to permit of any useful deduction being founded on deviations from an assumed healthy ratio. The amount of purin bodies in the food—a variable extrinsic factor—chiefly determines the

¹ *Deutsche med. Wochenschr.*, 1894. ² *Zeitschr. f. klin. Med.*, 1899.

³ *Loc. cit.*

⁴ *Münchener med. Wochenschr.*, 1899.

⁵ *Berlin. klin. Wochenschr.*, 1899.

⁶ *Zeitschr. f. klin. Med.*, 1901.

⁷ *Zeitschr. f. klin. Med.*, 1901.

⁸ *Centralbl. f. Stoffwechsel-u. Verdauungskrankh.*, 1900.

⁹ *Zeitschr. f. physiol. Chem.*, 1903.

proportion of uric acid in the urine. Under ordinary conditions as regards food and in an individual in his usual health, the urea-uric acid ratio may range from 1:30 to 1:50; in a daily estimation lasting over fifty days, in a healthy adult, Luff¹ found the ratio to vary from 1:28 to 1:55. Even special diet, as regards excess or absence of purin bodies, produces very unequal effects according to the idiosyncrasy of the individual. Those who habitually excrete large amounts of uric acid are much more easily influenced by diet than those whose daily output is small. Salkowski² points out the necessity of ascertaining the "personal equation" in each individual before attempting to draw any inference from what may appear to be an exceptional deviation from an assumed normal standard. It is better, therefore, to express the variations in uric acid excretion in positive rather than in relative terms.

DETECTION AND ESTIMATION OF URIC ACID.

The presence of uric acid is easily demonstrated by means of the *murexid test*, so named from an ancient purple dye which was obtained from a gastropod mollusc of the genus *Murex*. To a fragment of uric acid in a porcelain capsule a couple of drops of nitric acid are added, and the capsule is heated on the water-bath until the mixture is evaporated to dryness. The residue, which along with other oxidation products contains alloxantin, will have a reddish or orange colour; if it be very pale the nitric acid has not produced sufficient oxidation, consequently a few drops more should be added and the mixture again evaporated to dryness. When the capsule is cold, a glass rod dipped in ammonia-water and then held close over the deposit produces a reddish-purple coloration due to the formation of murexid or ammonium purpurate. On adding a drop of a solution of caustic soda, the colour becomes more blue, and disappears when the capsule is warmed; this distinguishes uric acid from several of the other alloxur bodies. The murexid test constitutes the only reliable reaction indicative of the presence of uric acid; it is exceedingly delicate and, when carefully applied, will detect the merest trace.

The *estimation of uric acid* may be made by various methods, as the Salkowski-Ludwig,³ the Haycraft⁴ (in both of which the uric acid is precipitated as a magnesio silver salt), the Gowland Hopkins⁵ (in which it is precipitated as ammonium urate), and innumerable

¹ *Loc. cit.*

³ *Wiener med. Jahrb.*, 1884.

² Virchow's *Archiv*, 1899.

⁴ *Brit. Med. Journ.*, 1885.

⁵ *Journ. of Path. and Bacteriol.*, 1893.

modifications of these and other methods. Both in respect to accuracy and to simplicity of procedure, the Gowland Hopkins is to be preferred to any of the processes which have hitherto been devised. It is carried out as follows :

To 100 c.c. of urine add 30 or 40 grms. of powdered ammonium chloride, and stir the mixture until the urine is saturated, which, after several minutes stirring, will be recognised by the presence of a small residue of undissolved chloride at the bottom of the vessel. The solution is allowed to stand for two hours, with occasional stirring to facilitate precipitation ; it is then passed through thin filter-paper and the precipitate is washed two or three times with a saturated solution of ammonium chloride. The filtrate should be perfectly clear. With a jet of hot water the precipitate is then washed off the filter into a beaker, and is heated, just to boiling, with excess of hydrochloric acid. The solution is allowed to stand two hours in the cold, whilst the uric acid separates and deposits ; the deposit is collected on a filter, the amount of filtrate, which should not exceed 30 c.c., being measured, and for each 15 c.c. 1 mgrm. should be added to the final result. The precipitated acid is then washed with cold distilled water ; it is afterwards washed off the filter with hot water, and warmed with sodium carbonate until dissolved, the solution being made up with water to 100 c.c. This is put into a flask and is mixed with 20 c.c. of sulphuric acid, and at once, whilst warm from the addition of the acid, is titrated with N/20 potassium permanganate solution, the flask being agitated. The end-reaction is indicated by the first appearance of a pink colour which lasts for an appreciable period. The permanganate solution is made by dissolving 1.578 grms. of potassium permanganate in a litre of distilled water : 1 c.c. = .00375 gm. of uric acid.

THE SALTS OF URIC ACID.

Uric acid is a weak dibasic acid which combines with metals so as to yield two, or possibly three, series of salts : the normal or neutral urate ; the biurate or acid urate ; and the quadriurate. When dealing with the combining properties of uric acid its formula may be thus expressed : $\text{H}_2(\text{C}_5\text{H}_2\text{N}_4\text{O}_3)$. In the normal urate, the whole of the displaceable hydrogen of the acid is replaced by the metal thus : $\text{M}_2\text{C}_5\text{H}_2\text{N}_4\text{O}_3$. In the biurate, one-half of the hydrogen is replaced : $\text{MHC}_5\text{N}_2\text{N}_4\text{O}_3$. In the quadriurate, one-fourth is displaced : $\text{MHC}_5\text{H}_2\text{N}_4\text{O}_3, \text{H}_2\text{C}_5\text{H}_2\text{N}_4\text{O}_3$. Normal urates do not exist in the living organism and may therefore be dismissed from consideration ; the biurates and the quadriurates alone claim our attention.

The **biurate** is the only stable salt of uric acid. Although it is called the acid urate its reaction is neutral, or, according to Tunnicliffe and Rosenheim,¹ is alkaline. It is less soluble than the normal urate, but is much more soluble than the free acid: sodium biurate is twelve times—or taking the solubility of uric acid as given by His—thirty-two times more soluble than uric acid. Biurates exist in two states: in the colloid or hydrate form, and in the crystalline form, into which the colloid form tends to pass (Ord²).

Bence Jones,³ following up Scherer's investigations on the constitution of amorphous urates, was the first to suggest the existence of a salt whose molecule consists of one molecule of uric acid with one molecule of sodium biurate, constituting the **quadriurate**, also called the **hemiurate** or **tetraurate**. W. Roberts⁴ carried these investigations further, and from them deduced a theory which he made a fundamental doctrine of the physiology and pathology of uric acid. According to this view, the quadriurate is the only form in which uric acid can be present in the blood and in the urine. The existence of the quadriurate is not universally admitted; many authorities still hold that uric acid is present in urine as the biurate. Roberts's theory requires both the uric acid in solution in urine and that which is contained in the deposit commonly called "urates," to be present in the form of the quadriurate. If some of the amorphous urates are collected, washed with alcohol and dried, and then a fragment is placed on a microscope slide with two or three drops of water, small crystals of uric acid are presently seen forming out of the amorphous salt. The quadriurate theory affords the explanation that when a quadriurate is treated with water it is decomposed into a molecule of biurate and a molecule of free uric acid. Tunnicliffe and Rosenheim,⁵ who regard the amorphous urates as a mixture of biurate and of uric acid in an amorphous form, hold that the biurate is dissolved out of the mixture and that a change takes place in the physical state of the uric acid portion of the mixture, which causes it to assume the crystalline form.

It is almost invariably stated that the quadriurate is a more soluble salt than the biurate, but there are good grounds for believing that the converse is the case. Assuming that, in solution, the excess of the feeble acid is not dissociated from the biurate, it is improbable that the "quadriurate" should be more soluble

¹ *The Lancet*, 1900.

² *The Influence of Colloids on Crystalline Form*, 1879.

³ *Journ. Chem. Soc.*, 1862.

⁴ *Croonian Lectures*, 1892.

⁵ *The Lancet*, 1900.

than the biurate itself, unburdened with an excess of a highly insoluble acid. The behaviour of many concentrated urines which, after standing some hours apparently unchanged, rapidly become turbid and then deposit urates, indicates something more than mere cooling of the urine; in such urine, at an earlier period, the addition of a drop or two of an acid immediately determines this change. Again, a specimen of clear, concentrated urine sometimes spontaneously deposits urates so copiously that, when subsequently warmed to the temperature of the body, all the deposit is not redissolved. Gowland Hopkins¹ states that when ammonium urate separates from a clear, acid urine, as an effect of adding neutral ammonium chloride, it is wholly in the form of biurate. These facts indicate that the uric acid which is in solution in the urine is in a more soluble form than that which is deposited as "quadriurates." It therefore seems probable that the uric acid is held in solution by the urine as a biurate, and that by interaction with the dihydrogen phosphates it is converted into a less soluble form. Experimental evidence, which would determine the solubility of the quadriurate, is not attainable on account of the rapidity with which the salt is decomposed in the presence of water.

Amorphous urates are composed of uric acid in combination with potassium, sodium, and ammonium; they appear as a yellowish or brick-red sediment which is deposited by febrile and other concentrated urines when they have stood for a time after being voided; if the urine is highly charged with uric acid salts the amorphous deposit may commence forming almost immediately. On account of the instability of the quadriurate in the presence of water, the deposit of amorphous urates, after standing awhile, tends to liberate free uric acid. Unpigmented urates are white; the colour displayed by those which are deposited from urine is chiefly due to uroerythrin, but other pigments also take part in its production. In young children, on account of the scarcity or the absence of pigmentary bodies in the urine, the deposit of urates is usually colourless, or nearly so. Urine which deposits urates has almost invariably an acid reaction; but it may be neutral. Such urines are clear when first voided; if allowed to stand in a glass vessel for some time after becoming turbid, a whitish-looking film will appear on the walls of the vessel when it is emptied; if the containing vessel be of white pottery-ware the coating will be pinkish. On gently warming urine that is turbid with urates, they are redissolved and the urine becomes clear.

¹ Schäfer's *Physiol.*, 1898.

XANTHIN or ALLOXUR BASES.

The terms "alloxur bodies" or "purin bodies" include uric acid along with a number of other closely allied substances; the terms "xanthin bases" or "alloxur bases" indicate these substances apart from uric acid.

The xanthin bases that have been found in urine are: *xanthin*, *hypoxanthin*, *guanin*, and *adenin*; by one or two investigators, *hetero-* and *para-xanthin*, *episarkin*, and *epiguanin* have also been found. The close relationship borne by these substances to uric acid is shown by the fact that xanthin contains one atom less oxygen than uric acid; hypoxanthin contains two atoms less; moreover, hypoxanthin may be converted in the system directly into uric acid. Adenin, xanthin, and probably guanin, exert the same influence as hypoxanthin on the excretion of uric acid (Krüger and Schmidt¹). As members of the group of purin bodies, the xanthin section bears an important relationship to some of the vegetable alkaloids, or methyl-purins—*e.g.*, theobromin, which is dimethyl-xanthin; and caffein, which is trimethyl-xanthin. The xanthin bases have feeble basic properties; and, with the exception of guanin, they readily dissolve in dilute acids and in ammonia.

Xanthin ($C_5H_4N_4O_2$) is a colourless substance which is insoluble in alcohol and ether; is but slightly soluble in cold water (1 : 14000), and is freely soluble in acids and alkalis. The daily amount present in normal urine does not exceed 2 or 3 centigrammes.

Hypoxanthin ($C_4H_4N_4O$), or *sarkin*, is much more soluble than xanthin in cold water (1 : 300), but, like xanthin, it is insoluble in cold alcohol and in ether. Hypoxanthin is present in normal human urine, but only in extremely small amount. In leucocythæmia the amount is increased.

Guanin ($C_5H_5N_5O$) is insoluble in cold water. It has been found in normal urine, and in increased amount in febrile urine.

Adenin ($C_5H_5N_5$) is less soluble than hypoxanthin in cold water (1 : 1086). It has not been found in normal human urine; but Stadthagen² found it in the urine of a leucocythæmic patient.

Reactions.—Xanthin does not respond to the murexid test. If, however, a little chlorine-water be used in addition to nitric acid, the test being in other respects performed as when testing uric acid, a similar reaction to that of the murexid test is obtained (*Weidel's test*). Hypoxanthin does not give this reaction; but, after being treated with hydrochloric acid and zinc, it gives a red coloration on

¹ *Zeitschr. f. physiol. Chem.*, 1902.

² *Virchow's Arch.*, 1887.

the addition of caustic potash. Like xanthin, guanin, when heated to dryness with nitric acid, leaves a light coloration which, on the addition of potash or soda, becomes orange-yellow—the *xanthin test*. A solution of adenin gives a red colour with ferric chloride.

The xanthin bases, like uric acid, have an endogenous source which is probably the nuclein of the tissue-cells, and an exogenous source furnished by the food-stuffs which contain nucleins and purins. The excretion of the xanthin bases takes place both by the urine and by the faeces. Burian and Schur¹ state that from 46 to 54 per cent. of the ingested purins (oxypurins) appear in the urine; Krüger² gives the urinary output as about one-third that of the faecal. On purin-free diet the daily urinary excretion of endogenous purins represents from 0.120 to 0.200 grm. of nitrogen; of this the xanthin bases furnish from 0.020 to 0.030 grm.; on a mixed diet the xanthin bases reach from 0.050 to 0.100 grm. (Walker Hall). These figures agree with those given by Burian and Schur, who state that the individual-constant in the greatest number of cases lies between 0.1 and 0.2 grm. of endogenous purin-nitrogen daily. As regards the faecal purins, Walker Hall³ gives the following determinations: On purin-free diet the faecal purin-nitrogen of a healthy man amounts to from 0.010 to 0.023 grm. daily, the xanthin bases amounting to from 0.025 to 0.0575 grm.; on a mixed diet the purin-nitrogen is increased to about 0.0265 grm. The value of the endogenous purins for the same individual, under the same life-conditions, remains fairly constant; but different individuals, although they live under similar conditions as to food and mode of life generally, may yield very different endogenous purin values.

The ratio borne by the xanthin bases to uric acid in normal urine is irregular; the mean of a number of estimations made by Krüger and Wulff⁴ gives the proportion of uric acid N to that of the other purin bodies (xanthin bases) in urine as 3.82 : 1; according to Walker Hall the ratio varies between 2.7 and 4.5 : 1. Krüger and Wulff state that the disturbances of the ratio are due to variations in the amount of xanthin bases rather than to variations in the uric acid. In some diseases, however, the ratio may be much more disparate, and its irregularity may be due to unwonted excess of uric acid.

The exogenous purins of the urine are derived from the purin bodies contained in food of which they represent the undecomposed remains; about one half the ingested food purin is excreted as exogenous purins in the urine, so that the amount of exogenous

¹ Pflüger's *Arch.*, 1901 and 1903.

² Virchow's *Arch.*, 1902.

³ *Brit. Med. Journ.*, 1903.

⁴ *Zeitschr. f. physiol. Chem.*, 1895.

purins is determined by the amount and kind of purin-containing food that is ingested, and, to some extent, by the existing activity of the digestive and assimilative powers of the system. Purins may be present in food in two conditions: as free purin, a condition which permits of easy solution in the digestive organs and consequently of rapid absorption; and as bound purin, the cleavage of which is slow and probably incomplete. Loewi¹ states that much of the bound purins are absorbed as such; a small portion is decomposed by the intestinal juice. Both free and bound purins occur in varying proportions in different kinds of food; glandular organs are rich in bound purins, that is, in nucleins. The commonest example of this type of food is sweetbread, which contains eight times as much bound purins as free; whereas the converse holds good with beef in which the free purins are sixfold more plentiful than the bound.

Some of the vegetable purins which are used as beverages rather than as foods exercise a pronounced influence on the output of the alloxur bases in urine, although their influence on the excretion of uric acid is very limited; this applies to the methyl-purins, caffein and, more especially, to theobromin (Krüger and Schmid²).

The individual factor of the endogenous purins may be estimated by putting the patient on, as nearly as possible, a purin-free diet, which may be composed of bread, butter, eggs, cheese, salad, green vegetables, potatoes, rice, sugar, and milk; no tea nor coffee should be taken. The amount of urinary purins is then determined, the result representing the endogenous factor; then fixed amounts of food which contains known percentages of purins are given, and the proportion of ingested to excreted purins is determined by calculation. The following table is abridged from Walker Hall³:

Codfish	.	.	0.0233	per cent. of purin nitrogen.
Salmon	.	.	0.0466	" " " " "
Tripe	.	.	0.0229	" " " " "
Mutton	.	.	0.0386	" " " " "
Veal	.	.	0.0465	" " " " "
Pork	.	.	0.0485	" " " " "
Ham	.	.	0.0462	" " " " "
Beef	.	.	0.0601	" " " " "
Chicken	.	.	0.0518	" " " " "
Sweetbread	.	.	0.4025	" " " " "
Rabbit	.	.	0.0380	" " " " "

See also Burian and Hall⁴ on *Die Bestimmung der Purinstoffe in tierischen Organen*.

¹ *Arch. f. ges. Physiol.*, 1901.

² *Zeitschr. f. physiol. Chem.*, 1901.

³ *Dissert. Vict. Univ.*, 1902.

⁴ *Zeitschr. f. physiol. Chem.*, 1903.

ESTIMATION OF URINARY PURINS.

The principal methods used for the isolation of the urinary purin bodies are: precipitation (a) with silver salts and (b) with copper salts. The silver method, first described by Salkowski, is, with numerous modifications, now generally used. The copper method, advocated by Krüger¹ and, with the acetate, by Pouchet,² is less accurate, and is not often used in its entirety. For clinical purposes it is sufficient to determine the total sum of the purin bodies in urine, including uric acid. The isolation of the individual bases is difficult and involves so much loss that it can only be successfully undertaken when large quantities of urine are dealt with; with small amounts, such as the clinical observer usually has at his disposal, the separation of the several bases is not practicable. This, of course, does not apply to uric acid, which can be separately estimated without difficulty.

Of the many modifications of the silver method, that of Camerer³ has certain advantages as regards the urine. It consists in preparing the urine by precipitating the phosphates with Ludwig's magnesium mixture, and then precipitating the purins by means of a solution of ammonio-silver nitrate: the silver chloride which is formed is dissolved by the ammonia and the silver purins remain as a precipitate. This is washed until it is ammonia-free; to ensure this, it may subsequently be boiled with magnesium oxide as recommended by Arnstein,⁴ after which the amount of nitrogen it contains is determined by Kjeldahl's process.

This method is not adapted for the everyday use of the clinical observer, who is therefore obliged to have recourse to simpler, though necessarily less accurate, means. For clinical use, Walker Hall's⁵ purinometer, on account of its simplicity and the ease with which it can be used, promises to be of great service; it affords a ready means of estimating by volume the amount of silver purins that have been precipitated after Camerer's method.

Two solutions are required: (1) consists of 100 c.c. of Ludwig's magnesia mixture (composed of magnesium chloride, 110 grms.; ammonium chloride, 110 grms.; ammonia, 250 grms.; and water to 1 litre); 100 c.c. of ammonia (20 per cent.) and 5 grms. of finely powdered talc (magnesium silicate). (2) Consists of 1 grm. of silver nitrate; 100 c.c. of strong ammonia; 5 grms. of finely powdered talc; and 100 c.c. of distilled water. (1) is used to precipitate the

¹ *Zeitschr. f. physiol. Chem.*, 1895.

² *Contrib. à la connaiss. de l'Urine*, 1880.

⁴ *Loc. cit.*

³ *Zeitschr. f. Biologie*, 1897.

⁵ *Loc. cit.*

phosphates; (2) precipitates the purins; the silver chloride that is formed is dissolved by the ammonia. The powdered talc is added in order to cause the otherwise gelatinous precipitate to rapidly subside and acquire a definite bulk.

The instrument (Fig. 6) consists of a stoppered tube graduated in cubic centimetres; by means of a stopcock the lower portion of the tube can be shut off. With the stopcock closed 90 c.c. of urine (which must be free from albumin) are poured in, and 20 c.c. of solution (1) added, and the instrument is inverted once or twice to promote admixture. The stopcock is then opened so as to allow the phosphates to subside into the lower chamber; when this has taken place the cock is once more turned off and solution (2) is added up to 100 c.c. The instrument is then freely inverted a few times so as to insure fine division of the precipitate of pale-yellow silver purin, and freedom in it from white particles of silver chloride; should any persist, a few drops of ammonia may be added. The purinometer is then placed in the dark for twenty-four hours, when the amount of the precipitate is read off. A table is furnished with each instrument, by means of which the percentage of nitrogen corresponding to the number of cubic centimetres of precipitate is at once seen. If

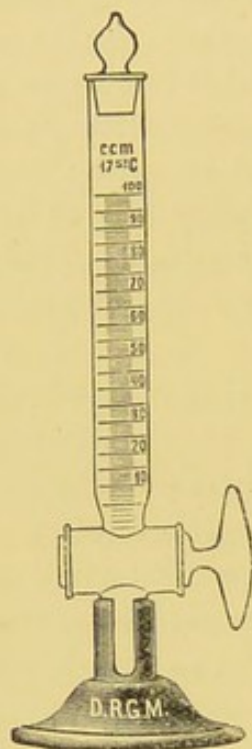


FIG. 6. Purinometer.

desired the contents of the tube can subsequently be filtered, and after being well washed and boiled with magnesia (to free it from ammonia) the precipitate may be subjected to Kjeldahl's process and its volume of nitrogen directly ascertained.

ALLANTOIN. $C_4H_6N_4O_3$.

Allantoin has been regarded as an end-product of proteid metabolism; more probably, however, it is an intermediate product between uric acid and urea. The close relation borne by allantoin to uric acid and to urea is thus shown:—When boiled with alkalies, allantoin is converted into allanturic acid and urea; the former yields hydantoic acid and parabanic acid, and in its turn parabanic acid yields oxaluric acid and urea. By the action of potassium permanganate uric acid is oxidised into allantoin and carbon dioxide, and by treatment with ammonium persulphate it yields allanturic acid, urea and glycol. If 6 parts of uric acid, 20 parts of ammonium persulphate

and 30 parts of ammonia are heated together to 36°C ., a brisk reaction takes place, at the end of which all the uric acid has disappeared; about 28 per cent. of it is replaced by allanturic acid and 42 per cent. by urea (Hugounenq¹).

Allantoin has been found in the urine of new-born children, of pregnant women, and also of men. It has been found in increased amount in diabetes insipidus and in hysteria (Pouchet²). When allantoin was administered in 1 to 2 grm. doses to human beings, Poduschka³ recovered from 30 to 50 per cent. unchanged in the urine.

Separation.—The urine is precipitated with baryta-water, and the filtrate is accurately neutralised with sulphuric acid and again filtered. After evaporating to commencing crystallisation, alcohol is added to the warm liquid; the alcoholic solution is decanted from the precipitate that is thrown down, and is then fully precipitated with ether. The combined precipitates, after being washed with hot alcohol, are dissolved in hot water from which crystals of allantoin separate on cooling (Meissner⁴).

Allantoin may be recognised under the microscope by the six-sided, prismatic form of its crystals, which are often clustered together as rosettes. It is easily soluble in hot water, but not in cold alcohol nor in ether. By prolonged boiling it reduces Fehling's solution. It gives the furfurol reaction like urea; but it does not respond to the murexid test.

¹ *Compt. rendus*, 1901.

² *Contrib. à la connaiss. de l'Urine*, 1880.

³ *Arch. f. exp. Pathol.*, 1901. ⁴ *Zeitschr. f. rat. Med.* [3], 24.

PIGMENTS AND CHROMOGENS.

A URINARY pigment is a substance which imparts colour to urine. A chromogen is a colourless, or nearly colourless, substance, which, in consequence either of the action of natural agencies, such as air and sunlight, or of chemical reagents, is capable of developing pigmentary properties. At present a satisfactory classification of these substances is scarcely attainable; it therefore appears preferable to group them together according to their clinical relations rather than to their chemical constitution.

(1) Three out of the four principal pigmentary substances of urine are derived from blood-pigment:—*urochrome*, *urobilin*, *hæmatoporphyrin*; *uroerythrin* is probably not derived from the blood.

Of these, *urochrome* and *uroerythrin* always appear as formed pigments; whilst *urobilin* and *hæmatoporphyrin* appear both as formed pigments and as chromogens.

(2) The following occur as chromogens and are not derived from blood-pigment:—*Indoxyl* and *skatoxyl* compounds, *urorosein*, *alkapton* (homogentisic acid), *melanin* (occasionally as pigment-granules).

In addition to these, *blood-pigments* and *bile-pigments* may be present in urine; also adventitious pigments derived from *fruits*, *drugs*, and other pigment-yielding substances.

Of the substances above named: *urochrome*, *urobilin*, *hæmatoporphyrin*, *uroerythrin* (?), *indoxyl* compounds, *skatoxyl* compounds (?), and *urorosein* may be present in normal urine.

UROCHROME.

Urochrome is the pigment to which urine owes most of its normal yellow colour; some abnormally high-coloured urines, which contain no excess of *urobilin* nor of other recognisable pigment, probably owe their depth of colour to excess of *urochrome*. The name was originally given by Thudichum¹ to a substance which he extracted from urine, and which he maintained was not derived from hæmoglobin. Garrod,² who

¹ *Brit. Med. Journ.*, 1864.

² *Bradshaw Lecture*, 1900.

has very fully investigated the nature and composition of the urinary pigments, retains the name urochrome, but applies it to a substance obtained in a different manner, and to which he attributes a different origin to that attributed by Thudichum; the description which follows is in accordance with the results obtained by Garrod. Urochrome is an iron-free product, which is very soluble in water, less so in alcohol, and is but slightly soluble in acetic ether and amyl alcohol; it is insoluble in ethyl ether, chloroform, and benzene. A solution of urochrome obscures the violet end of the spectrum, but it yields no absorption bands; it further differs from urobilin in the absence of fluorescence when treated with zinc salts. With nitric acid it gives a reaction similar to the xantho-proteic reaction, and it is precipitated from solution by phosphotungstic acid. Klemperer¹ shows that it is extracted from urine by animal charcoal, and points out its high molecular, colloidal nature, which entirely prevents any dialytic diffusion. He believes that urochrome is a direct derivative of the colouring matter of blood, and that it is formed in the kidneys.

The relationship of urochrome to urobilin (and consequently to hæmoglobin) has been demonstrated by the reciprocal conversion of each into a substance which appears to be identical with the other. By treating urobilin with potassium permanganate, Riva and Chiodera² obtained a substance which yields the negative reactions of urochrome: it gives no absorption band, nor does it fluoresce with zinc salts. By treating an alcoholic solution of urochrome with aldehyde, Garrod³ obtained a substance which yields the reactions of urobilin: it gives the characteristic absorption band and fluoresces with zinc salts; the substance obtained from urobilin by the action of potassium permanganate behaves in the same way. As pointed out by Garrod, this reaction of urochrome with aldehyde affords a delicate test for urochrome; it only takes place, however, when the urochrome is in alcoholic solution; the addition of water arrests it. The reaction is not due to the aldehyde itself, but to some substance formed in it when it has been exposed to light and warmth.

Separation.—Garrod's method⁴ of isolating urochrome is as follows: The urine is saturated with ammonium sulphate and after standing is filtered; the filtrate is then shaken with about one-fifth its volume of absolute alcohol, which quickly separates from the saline solution, and carries with it some of the colouring matter, which can be almost entirely removed by repeated extraction. The extract, after being diluted with a large volume of water, is saturated with ammonium sulphate, by which the alcohol is again separated along with the

¹ *Berliner klin. Wochenschr.*, 1903.

² *Arch. Ital. di Clin. Med.*, 1896.

³ *Journ. of Physiol.*, 1897 and 1903.

⁴ *Proc. Roy. Soc.*, vol. lv., 1894.

pigment in a purer condition. This alcoholic solution is faintly alkalisied with ammonia and is evaporated to dryness; after being well washed with acetic ether the product is dissolved in alcohol by prolonged digestion. The alcoholic solution is evaporated down until it has acquired a deep orange colour, and is then poured into an equal volume of ether; this determines the precipitation of the urochrome as an amorphous brown substance.

Klemperer¹ separates the urochrome by shaking the urine, until it is colourless, with finely-powdered animal charcoal which takes up the colouring matter. The animal charcoal is then washed to remove any indican, and, when dry, is extracted with alcohol in a Soxhlet's apparatus, the alcoholic extract being afterwards dealt with as in Garrod's method.

UROBILIN.

Urobilin, discovered by Jaffe² in 1868, exists in normal urine almost wholly as a chromogen; sometimes the pigment itself is present, and in pathological urines it is very common. MacMunn and others have assumed the existence of two kinds of urobilin—normal and pathological; it has been proved, however, that urobilin from all sources is one and the same substance. When much urobilin pigment is present the urine is dark-coloured, and resembles bile-stained urine, especially as the froth produced by shaking the urine has a bright yellow colour. When in this amount, the presence of urobilin may be recognised by adding to a little of the urine in a test-tube half a dozen drops of a 10 per cent. solution of zinc chloride, followed by as much ammonia as is necessary to dissolve the precipitate produced by the zinc salt; a more or less distinct green fluorescence results, which is best seen by allowing the light to fall sidewise on the tube whilst it is held against a black surface. Urobilin is soluble in all the usual solvents. In acid solution it gives one absorption band where the green merges into the blue of the spectrum, between b and F, passing a little beyond the latter.

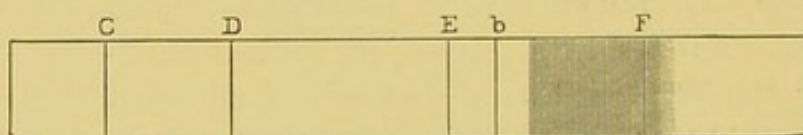


FIG. 7. Absorption spectrum of urobilin.

Urobilin forms compounds with various metallic salts, the spectra of which vary slightly from the spectrum given by the free pigment; in its metallic spectrum with zinc the band appears somewhat nearer to E; it is not easily seen, however, unless much urobilin and but little

¹ *Loc. cit.*

² *Centralbl. f. med. Wissensch.*, 1868.

ammonia are present. With some metals—*e.g.*, calcium—it yields no band. Urobilin gives the biuret reaction.

In 1871, Maly¹ discovered that by acting on bilirubin with sodium amalgam he obtained a product—hydrobilirubin—closely resembling urobilin, with which, by some, it is held to be identical; it presents certain differences, however, and probably occupies an intermediate position between bilirubin and urobilin. Recently, Nencki and Zaleski,² by reducing acethæmin with iodic acid and phosphonium iodide, obtained a product—hæmopyrrol—which, when exposed to the air, undergoes spontaneous oxidation into urobilin. These discoveries furnish the link between hæmoglobin and urobilin, and demonstrate the derivation of urobilin from the colouring matter of blood. The identity of urobilin with stercobilin, the chief pigment of the fæces, is beyond all doubt; therefore the latter term is superfluous—urobilin is the chief pigment both of the fæces and of the urine. Two views are held as to the path taken by hæmoglobin in its downward course to urobilin: one, regards bilirubin as an intermediate product that, by bacterial agency, is reduced in the intestines to urobilin; the other, derives urobilin directly from hæmoglobin, without the intervention of the liver. No bile-pigment is present in normal fæces; it is replaced by urobilin. F. Müller³ found that occlusion of the common bile duct causes disappearance of urobilin from fæces and urine; he also found that if, whilst the bile duct is occluded, pig's blood is introduced by means of a tube into the patient's stomach, urobilin appears both in the fæces and in the urine, and that it does not disappear from the urine until the fæces are free from it. When unaltered bile-pigment is present in the fæces, as it is under certain abnormal conditions, urobilin is absent, or nearly so. Vaughan Harley⁴ found that frequently repeated doses of calomel cause the motions to assume a green colour, and to contain large quantities of bile-pigment and only small quantities of urobilin; these effects are due to diminution of the intestinal bacteria by which, under ordinary conditions, the biliverdin is converted into urobilin. It is probable then, as the result of bacterial action, that bile-pigment yields urobilin, the change mostly taking place in the colon. From these observations it is to be assumed that the urinary urobilin is chiefly derived from the intestinal pigment; some is possibly furnished directly from the blood, by the liver which retains the iron of the hæmoglobin, and some from other tissues

¹ *Centralbl. f. med. Wissensch.*, 1871.

² *Berichte d. deutsch. chem. Gesellsch.*, 1901.

³ *Schlesische Gesellsch. f. Vaterl. Kult.*, 1892.

⁴ *Brit. Med. Journ.*, 1896.

in which hæmoglobin is reduced without the intervention of the liver.

As previously stated, urobilin is represented in normal urine chiefly by its chromogen, which has been termed urobilinogen. This chromogen is very susceptible to the action of light, by which it is converted into urobilin. If freshly passed urine is kept from daylight and, after being acidulated with acetic acid, is extracted with acetic ether, the chromogen is transferred to the ether; if the ether-extract be shaken with water it dissolves no colouring matter; but on exposure to direct sunlight—the violet rays being more active than the red—the water becomes coloured and yields the spectrum of urobilin (Saillet¹).

The daily amount of urobilin in normal urine varies from a mere trace to 15 or 20 mgrms. Saillet found much more—from 30 to 130 mgrms.—including that which is present as a chromogen. G. Hoppe-Seyler² found from 0.08 to 0.14 grm.

Under pathological conditions, the amount of urobilin in the urine has been found to be increased in diseases which are accompanied by excessive intestinal putrefaction, and by stoppage of the action of the bowels, especially when accompanied by fever; in perityphlitis and in pyæmia enormous amounts have been found. In pneumonia, in some of the infectious fevers, scarlet fever, small-pox, and in malaria it is increased. In conditions accompanied by hæmorrhage into the intestinal tract, and elsewhere; hæmorrhagic purpura, scurvy, hæmatothorax, infarcts of the lungs and other organs, malignant disease of the peritoneum with effusion of blood into its cavity, rupture of a cerebral vessel, and large subcutaneous or deeper-seated hæmorrhages of idiopathic or traumatic origin, excess of urobilin occurs. As first pointed out by Mott³ and Hunter,⁴ urobilinuria is persistently present in pernicious anæmia; it occurs also in other conditions associated with hæmolysis. In cirrhosis and in cancer of the liver large quantities of urobilin are constant; in Addison's disease and in chronic plumbism there may be excess. Blood poisons, such as antifebrin, antipyrin, and sulphonal, naturally cause an increase. On the other hand, blocking of the common bile duct by stone or tumour, severe phosphorus poisoning, and acute yellow atrophy of the liver, diminish or inhibit the formation of urobilin (Riva⁵). The amount of urobilin in the urine is either not increased, or is diminished in simple anæmia, in leucocythæmia, in starvation, in diphtheria, and in Bright's disease; albuminuria and

¹ *Revue de Méd.*, 1897.

² *Virchow's Archiv*, 1891.

³ *The Lancet*, 1889.

⁴ *The Practitioner*, 1889.

⁵ *Arch. Ital. di clin.* .., 1898.

urobilinuria rarely occur together. Morfaux¹ states that when the kidneys keep back methylene-blue they also keep back urobilin.

When the quantity of urobilin in the urine is very great—and in some cases it has reached as much as 0.44 grm. in the day—a yellow colour of the conjunctivæ and the skin often occurs, to which Gerhardt gives the name “urobilin-icterus.”

Separation.—For routine clinical purposes the simplest way is to fill nearly half of a large test-tube with urine, and, after acidulating with a few drops of acetic acid, to extract it with an equal volume of acetic ether; this requires care, as the ether and the urine very readily form an emulsion. After closing the tube with the thumb, it should be alternately inverted and restored to its original position as frequently as may be deemed necessary, a pause being made after each inversion so as to allow the ether to ascend through the column of urine. If, notwithstanding all care, some emulsification takes place, the extract (after separation) may be cleared with a little ethyl alcohol, or with the aid of the centrifuge. Amyl alcohol may be substituted for acetic ether, and in some respects is preferable; if an emulsion forms it should be poured on a filter moistened with amyl alcohol, by which it is separated into two clear layers, the alcohol floating on the urine. (Huppert.) The presence of urobilin in the ethereal or the alcoholic extract is recognised by the spectroscope, and by adding a few drops of a saturated solution of zinc acetate in ethyl alcohol; this produces a green fluorescence which, if much urobilin be present, is extremely brilliant. For the fluorescence to be seen at its best the solution must be perfectly limpid; a few drops of ammonia will probably remove any turbidity.

If bile-pigment is also present, it may be removed as described in the following paragraph, or by Bouma's² method. To 8 c.c. of the urine, 2 c.c. of 10 per cent. solution of calcium chloride are added, and some very weak solution of ammonia is dropped in until the reaction is faintly acid; it must not become alkaline, else the urobilin will be precipitated along with the bile-pigment. The precipitate is separated in the centrifuge, and the clear liquid is tested for fluorescence by the addition of zinc acetate.

The isolation of urobilin for experimental purposes may be accomplished by a modification of Garrod and Hopkins's³ method. The urine is prepared by precipitation with one-third its volume of a mixture of one volume of saturated barium chloride solution and two volumes of saturated barium hydrate solution in order to remove bile-pigments and hæmatoporphyrin, along with the uric acid; after

¹ *Comptes rend. de la Soc. Biol.*, 1899.

² *Festbundel*, Talma, 1901.

³ *Journ. of Physiol.*, 1896.

filtration the excess of barium is removed by precipitation with a concentrated solution of sodium sulphate, and is nearly neutralised with sulphuric acid (Fr. Müller¹). The filtrate from this is saturated with ammonium sulphate, by which the urobilin is thrown down; the precipitate is collected on a filter and is dried. It is then extracted with large quantities of water, from which the urobilin is again precipitated by saturation with ammonium sulphate. The final precipitate, when dry, is extracted with absolute alcohol.

Garrod and Hopkins recommend another method which is useful when only a small amount of urobilin is present. The urine is saturated with ammonium chloride in order to remove the urates; after filtration, and acidulation with sulphuric acid, the filtrate is saturated with ammonium sulphate and is then extracted with an equal volume of a mixture of one part chloroform and two parts ether. After separation the extract is shaken with a little water to which the urobilin is transferred; in order to facilitate this, a trace of alkali may be added so as to neutralise any acid that the ether-chloroform may have taken up. If it is requisite to obtain the urobilin in a very pure state it must be reprecipitated from the aqueous solution by ammonium sulphate and extracted as before.

HÆMATOPORPHYRIN.

This substance, first discovered in urine by MacMunn in 1880, is an iron-free decomposition-product of hæmatin, from which it has been artificially prepared by Nencki and Sieber.² It is soluble in acids and alkalies, in ethyl and amyl alcohols, chloroform, and acetic ether. Hæmatoporphyrin yields very characteristic spectra, which vary according to its reaction—acid or alkaline—and its basic combinations. The acid spectrum consists of a narrow line, one border of which touches the D line (Fig. 8), the other border extending a short distance towards C, and a broader and better-defined band situated nearly midway between D and E, which shades off towards D. In alkaline solution hæmatoporphyrin yields a four-banded spectrum: a narrow, well-defined band midway between C and D; a weaker band, a short distance from D in the direction of E; a stronger-marked band, also between D and E, its far border touching E; and a broad, dark band which reaches from b to F. The neutral spectrum resembles a combination of the acid and alkaline spectra. Sometimes a five-banded spectrum occurs in alkaline or in neutral solution; the fifth is a feeble band, close to the violet side of

¹ Neubauer and Vogel, 1898.

² *Berichte d. deutsch. chem. Gesellsch.*, 1884.

the band in the red of the alkaline spectrum. Hæmatoporphyrin enters into a peculiar combination with metals, the resulting spectrum, called its metallic spectrum, closely resembling the spectrum of oxyhæmoglobin; the hæmatoporphyrin that may be carried down by the deposition of amorphous urates yields the metallic spectrum.

When hæmatoporphyrin is extracted from urine that has a naturally acid reaction it yields the alkaline spectrum; and further, if to normally acid urine, hæmatoporphyrin which gives the acid spectrum be added, its spectrum at once takes the alkaline type. These somewhat paradoxical results are probably due to the hæmatoporphyrin combining with some of the bases of the phosphates, to which the acidity of the urine is due.

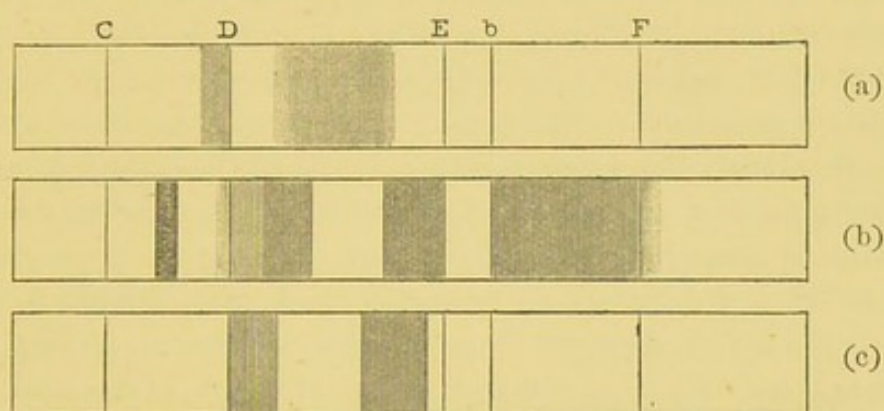


FIG. 8. Absorption spectra of Hæmatoporphyrin—(a) acid; (b) alkaline; (c) metallic.

Hæmatoporphyrin in very small amount is present in normal urine (Garrod¹), and also in the fæces. Most of the hæmatoporphyrin present in the urine is derived from the blood-pigment of the patient; it is stated that some may be derived from the blood-pigment contained in animal food, and even from the colouring matter of vegetable food, some kinds of which yield a pigment—phylloporphyrin—derived from chlorophyll, which is nearly allied to hæmatoporphyrin (Stokvis²). Along with the formed pigment its chromogen is also present in urine. The daily amount of hæmatoporphyrin in normal urine amounts to from 4 to 8 mgrms.

Hæmatoporphyrin is probably formed in the liver; almost any disorder of the function of that organ is attended by an increased amount of the pigment in the urine. The spleen does not appear to play any part in its production; in the urines from three cases of splenectomy Garrod³ found only the ordinary traces of hæmato-

¹ *Journ. of Physiol.*, 1892.

² *Zeitschr. f. klin. Med.*, 1895; *Nederl. Natuur-en Geneesk. Congres*, 1899.

³ *The Lancet*, 1900.

porphyrin. The source of hæmatoporphyrin naturally leads to the inference that it would be present in the urine in increased amount under all conditions which are accompanied by excessive hæmolysis; this, however, is not necessarily the case. Hopkins¹ has shown that in pernicious anæmia no more hæmatoporphyrin is present than may be found in normal urine. In two or three cases I have found more than in normal urine, but not so much as in many cases of slight hepatic derangement without obvious blood-changes.

Solutions of hæmatoporphyrin have a purple-red colour; if very dilute the colour is pink. Urine that contains an excess of hæmatoporphyrin may have a reddish tinge; but, not unfrequently, its colour differs little, if any, from that of normal urine. When hæmatoporphyrinuria is due to drugs, such as sulphonal, the urine is usually dark in colour, like burgundy; this, as shown by Hammarsten,² is not due to the hæmatoporphyrin, but to some other unknown pigment or pigments; when all the hæmatoporphyrin has been extracted, the urine remains much the same colour as before. A few instances have been recorded in which burgundy-red urine containing hæmatoporphyrin has been excreted by people who have not taken any drugs; they comprise cases of tuberculosis, enterica, obscure nervous diseases [sulphonal?], hydroa æstivale (Harris³), gastric ulcer with hæmatemesis, and one remarkable case, recorded by Nebelthau,⁴ of a woman who was the subject of congenital syphilis, and who had passed red urine as long as she could remember.

The pathological conditions under which hæmatoporphyrinuria has been met with comprise cases of carcinoma, cirrhosis, and fatty changes in the liver; mitral disease of the heart in which the liver is enlarged; febrile diseases, such as enterica, pneumonia, acute rheumatism, and gout; septic diseases; phthisis and other forms of tuberculosis; Addison's disease; Hodgkin's disease; chronic syphilis and chronic plumbism. After the prolonged use of certain drugs which act as blood poisons, such as sulphonal and trional, hæmatoporphyrinuria, with dark-red urine, has frequently been observed. Hæmatoporphyrin, in more than the physiological amount, is by no means infrequently present in the urine of those who exhibit no perceptible deviation from health; in other instances it accompanies insignificant derangements of a non-febrile character. Pål⁵ records a unique case of paroxysmal hæmatoporphyrinuria in a man aged sixty-six, who, after exposure to cold, passed urine which contained hæmatoporphyrin without any hæmoglobin or blood corpuscles. The

¹ *Guy's Hosp. Reps.*, 1893.

² *Skand. Arch. f. Physiol.*, 1891.

³ *Brit. Med. Journ.*, 1898.

⁴ *Zeitschr. f. physiol. Chem.*, 1899.

⁵ *Centralbl. f. innere Med.*, 1903.

patient had repeated attacks which, in etiology and course, including local asphyxia, corresponded with those of paroxysmal hæmoglobinuria. The liver was somewhat enlarged; the spleen not materially so. The patient had had syphilis.

The amount of hæmatoporphyrin present in urine under pathological conditions has only exceptionally been determined; in a litre of urine from a case of sulphonal hæmatoporphyrinuria Salkowski¹ found 0.87 grm.

Detection and Separation.—It is extremely rare for the urine itself to yield the spectrum of hæmatoporphyrin; some method of separation, therefore, has to be adopted. For clinical purposes, when it is desired to ascertain if hæmatoporphyrin is present in the urine in an amount that is beyond the normal trace, it will be sufficient to acidulate some of the urine with a few drops of acetic acid, and then to extract it with acetic ether or amyl alcohol, as described in the extraction of urobilin. Notwithstanding the presence of the acetic acid, the extract thus obtained gives the alkaline spectrum; this is accounted for by the fact that organic acids do not act upon hæmatoporphyrin in such a way as to cause it to give its acid spectrum; the mineral acids only do this.

The separation of hæmatoporphyrin from urobilin—both pigments if present being extracted from urine by the above method—is not easily accomplished. Sallet² recommends shaking the acid ethereal extract with water which takes up the urobilin, but not the hæmatoporphyrin; or, by shaking the ethereal extract with 5 per cent. hydrochloric acid, which takes up both pigments; then, after separation, the dilute acid is alkalisied with ammonia, is re-acidulated with acetic acid and shaken with sulphuric ether, by which the hæmatoporphyrin is extracted, and the urobilin is left behind. In clinical work separation is unnecessary: the spectroscope demonstrates the presence of hæmatoporphyrin, and the fluorescence produced by the subsequent addition of a zinc salt the presence of urobilin.

When hæmatoporphyrin is present in little more than a trace, as in normal urine, Garrod's³ method of separation is the best. To every 100 c.c. of the urine add 20 c.c. of a 10 per cent. solution of sodium hydrate; by this treatment the phosphates are precipitated, and they carry down with them the hæmatoporphyrin. The precipitated phosphates are collected and, after being washed, are dissolved in alcohol to which a sufficiency of hydrochloric acid has been added to effect their solution; the solution thus obtained gives the spectrum of acid hæmatoporphyrin. On the addition of

¹ *Zeitschr. f. physiol. Chem.*, 1891.

² *Revue de Méd.*, 1897.

³ *Journ. of Physiol.*, 1895.

ammonia the phosphates are reprecipitated, along with the hæmatoporphyrin, as at first, in the alkaline state; with the aid of a little acetic acid they are redissolved and, after dilution with water, the solution is extracted with chloroform, which then gives the spectrum of alkaline hæmatoporphyrin because, as previously explained, organic acids do not produce the acid spectrum. By evaporating the chloroform-extract, a red-coloured deposit of hæmatoporphyrin is left.

In the burgundy-red urine from cases of chronic sulphonal and trional poisoning, both the hæmatoporphyrin and the accompanying pigment, to which the urine owes its deep colour, resist extraction by acetic ether, chloroform, amyl alcohol, and the other usual solvents; nor, usually, is Garrod's method of precipitating the hæmatoporphyrin along with the phosphates more successful. The probable explanation is that in sulphonal urine most of the pigment exists in metallic combination. With such urines Salkowski's¹ method may be resorted to. About 30 c.c. of the urine is precipitated with a solution which consists of equal volumes of a saturated solution of barium hydrate and of a 10 per cent. solution of barium chloride; the precipitate is washed with water and then with absolute alcohol. The moist precipitate is rubbed in a mortar with six to eight drops of hydrochloric acid and as much alcohol as to make a thin gruelly liquid; this is filtered through a dry filter, and the rose-coloured filtrate gives the spectrum of acid hæmatoporphyrin. By alkalisising with ammonia the alkaline spectrum is obtained; any turbidity caused by the ammonia may be removed by the addition of a little water or by filtration. The solution thus obtained contains other pigments besides hæmatoporphyrin, but its spectrum can easily be recognised.

In a case of hæmatoporphyrinuria, not due to sulphonal, in which the urine was dark coloured, Calvert and Garrod² found that acetic ether took up but little of the pigment, which, however, was precipitated, along with the phosphates, by alkalisising the urine. The purple-coloured pigment which remained in solution after the removal of the hæmatoporphyrin was thrown down by barium chloride and, on treatment with dilute sulphuric acid, yielded a red-coloured solution which gave no absorption band; alkalisation destroyed the red colour which was restored by acidulation. This substance was soluble in water and in acetic ether; but it was insoluble in absolute alcohol, amyl alcohol, and chloroform.

¹ *Zeitschr. f. physiol. Chem.*, 1891.

² *Trans. of the Clinical Soc.*, 1901.

UROERYTHRIN.

Uroerythrin is a very common pigment in urine, but whether it is a normal constituent or not is doubtful; when present it is as a pigment and not as a chromogen. It possesses powerful pigmentary properties and is the principal agent that imparts the well-known pink or red colour to amorphous urates and uric acid, for which it has the strongest affinity; uroerythrin is deficient in the urine of childhood, hence the unpigmented urates. Uroerythrin is soluble in water, ethyl, and amyl alcohols, acetic ether, and chloroform. Its solutions have a deep orange-colour which is in contrast with the pink tint of urates. Garrod¹ suggests that the pigment forms a combination with the urates, and cites as evidence that urates have a distinct spectrum of their own, which differs from the spectrum of uroerythrin in solution; moreover, uroerythrin cannot be extracted from the ordinary urates by one of its solvents, such as ethyl alcohol. When in solution, uroerythrin is quickly bleached by the action of sunlight, and is unstable when kept in the dark. Alkalies change the colour of the pigment to green, as may be observed on adding a little solution of potash to some pigmented urates; this reaction distinguished uroerythrin from all the other urinary pigments. Uroerythrin is not fluorescent. It gives a two-banded spectrum,

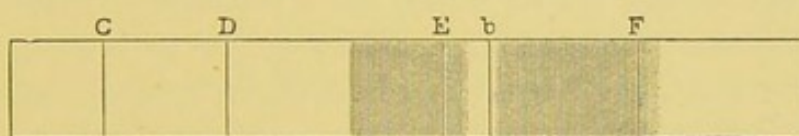


FIG. 9. Absorption spectrum of uroerythrin.

first accurately described by MacMunn,¹ one band of which commences about half-way between D and E (Fig. 9) and extends a little beyond E; the second band is in the position of the urobilin band—between b and F, beyond which it extends somewhat. The bands are ill-defined and are connected by a less degree of light-absorption.

Uroerythrin is probably not a derivative of hæmoglobin, although its presence in urine appears to be largely dependent on the functional activity of the liver; any simple disorder of that organ is sufficient to cause an excessive amount of uroerythrin to be present in the urine. In many organic diseases of the liver, such as cirrhosis and malignant disease, the amorphous urates that are deposited from the urine are most brilliantly coloured, with a bright red tint, which is quite different from the subdued pink of the

¹ *Proc. Royal Soc.*, 1883.

ordinary urates. Uroerythrin is present in excess in cases of chronic heart disease accompanied by hepatic enlargement; in acute rheumatism and in gout; in influenza, malaria, and other febrile conditions, though in some, as enterica (Zoja¹), it is unusual to find any large amount. On account of the hepatic derangements produced, gluttony and the abuse of alcohol determine an increase, as do also excessive sweating and muscular exertion. Cerebral hæmorrhage, acute pulmonary affections, and organic abdominal diseases have been observed to be attended by the presence of much of the pigment in the urine. On the other hand, in Bright's disease it is usually absent. Riva² found that, in cases of cirrhosis of the liver, the amount of uroerythrin could be greatly diminished by putting the patient on a milk diet; he also states that excess of uroerythrin is usually accompanied by excess of urobilin.

Separation.—Urine that contains much uroerythrin has a noticeable reddish-orange colour, which distinguishes it from ordinary urine; even when the colour does not present any abnormality, a peculiar pink line may be observed round the margin of the clear urine, best seen by tilting the chamber vessel and viewing its contents with the light falling obliquely on them from above. When a thick layer of urine which contains much uroerythrin is examined with the spectroscope, aided by a good light, the spectrum of the pigment may be seen; it is usually necessary, however, to extract the urine with amyl alcohol before using the spectroscope. The first band only (between D and E) can be relied on as indicative of uroerythrin; the second band is common to it, to urobilin, and to hæmatoporphyrin.

In order to obtain a pure product it is best to follow Garrod's³ method, which is founded on the fact that, whilst spontaneously deposited urates do not give up their uroerythrin to ethyl alcohol, those which are artificially precipitated do so readily. Pigmented urates in large amount are washed with cold water, and, whilst moist, are dissolved in warm water and then precipitated with a saturated solution of ammonium chloride; the precipitate is washed with saturated ammonium chloride solution (to remove urobilin) until the washings are free from colour. The filter, with the precipitate, is digested with warm alcohol for several hours in the dark; then, after filtration, the alcoholic extract is diluted with twice its volume of water and is shaken with successive portions of chloroform in order to remove hæmatoporphyrin and other impurities. Some fresh chloroform is now added along with a drop or two of

¹ *Arch. Ital. di clin. Med.*, 1893.

² *Gaz. Med. di Torino*, 1892.

³ *Journ. of Physiol.*, 1895.

acetic acid, which, when shaken, dissolves the uroerythrin. After separation, the chloroform is washed with water and is then allowed to evaporate in a warm place in the dark. The solid residue is soluble in absolute alcohol.

INDOXYL COMBINATIONS.

Indoxyl is a product of the oxidation of indol which, when conjugated with sulphuric acid, forms indoxyl-sulphuric acid, known as urinary indican. The name "indican" was first given by Schunck¹ to a substance he discovered in woad (*Isatis tinctoria*), which he showed was the indigo-producing body of that plant. In normal human urine he afterwards found an indigo-yielding substance which he held to be identical with indican; the two substances, however, have not the same chemical constitution: indican is a glucoside, the urinary product is not. Indoxyl-sulphuric acid is not present in urine in the free state, but in combination with bases, chiefly as potassium indoxyl-sulphate. Some of the indoxyl present in urine is conjugated with glycuronic acid, as indoxyl-glycuronic acid which, in combination with bases, forms salts that are very unstable. The indoxyl-compounds being derived from indol—a product of the putrefactive processes which, in a greater or lesser degree, accompany proteid digestion in the intestine—indicate by their amount in the urine the intensity of the intestinal decomposition. The intestine of the newly-born infant is free from micro-organisms, consequently indican is absent from the urine. The normal urine of adults contains from 1 to 6 mgrms. of indoxyl salts to the litre; if large quantities of animal food are eaten the amount is greater.

The amount of indoxyl compounds in urine may be enormously increased under various pathological conditions: amongst them are all conditions in which the intestinal putrefactive processes are excessive, whether due to retention of the intestinal contents or to catarrhal and other disorders which depress the digestive functions and promote decomposition, even though attended by diarrhœa. The proteid material, the decomposition of which furnishes the indol, is chiefly present in the small intestine; hence, arrest of the contents of the small intestine, and abnormal conditions of its digestive function, are especially active factors in the formation of indol (Jaffe²). In ileus, and in tuberculous intestinal disease, enormous quantities of indoxyl compounds are usually present in the urine; in intestinal catarrh, in hæmorrhage into the higher parts of

¹ *Mem. Manchester Lit. and Phil. Soc.*, 1857.

² *Pflüger's Arch.*, 1870.

the intestine, in purpura hæmorrhagica, in gastric catarrh and ulcer, in dilatation of the stomach, in malignant disease of the stomach and intestinal canal, in various disorders and diseases of the liver, and in Addison's disease, excess usually occurs. Any localised inflammatory or other disorder of the abdominal viscera, by which peritonitis is set up, invariably has the same effect. A minor degree of so-called indicanuria is often present in neurotic patients who suffer from neurasthenia; in these cases the intestinal digestion is usually sluggish, and it is possible that other unrecognisable products of proteid decomposition may be formed by the same processes that lead to the formation of indol in excess, and that they may be answerable for some of the psychical symptoms—mental depression and lassitude—which form such a prominent feature in the clinical history of the condition in question. There are reasons to believe that the functional activity of the liver exercises an influence upon the amount of indol which reaches the circulation; normally, the liver probably keeps back much indol which in a diseased or inactive condition it allows to pass unchanged; the same observation is applicable to toxins of intestinal origin. It is sometimes stated that simple constipation does not increase the amount of indican in the urine; this can only be accepted as a relative statement, since in most cases such a condition does lead to an increase, though not excessive, as in organic obstruction. It is to be observed that idiosyncrasy exercises great influence in determining the amount of indoxyl compounds in healthy urine; the urine of some perfectly healthy individuals regularly contains an amount that would be pathological in others.

The intestinal tract is not the only source of urinary indican; indol may be formed by bacterial agency being brought to bear on proteid substances, detached from the general circulation, in any part of the body. In empyema and other collections of pus which have become septic, in quinsy and all septic abscesses, in bronchiectasis, in pulmonary tuberculosis—especially when cavities exist in the lungs—apart from any implication of the intestines, and in pyonephrosis much indican has been found in the urine.

As stated by von Noorden,¹ it is possible that some of the indican may be derived from proteid substances furnished by the system under ordinary physiological conditions; the mucus and other compound proteids which enter into the composition of the intestinal juices and of the bile may furnish a certain *quotum*. In inanition indican is absent, or nearly absent, from the urine; any that is present is probably derived from the above-named sources.

¹ *Pathol. d. Stoffwechsels*, 1893.

When an excess of indican is present in urine, the amount of phenol is also increased. On the other hand, phenol may be in excess without any increase in the amount of indican; this is likely to occur when the excess of phenol is derived from collections of pus that are undergoing bacterial putrefaction, apart from immoderate intestinal decomposition.

Detection and Estimation.—Indoxyl compounds are present in urine as chromogens, and, consequently, they do not affect its colour, although, from other causes, urine which contains them in excess is frequently dark in colour. So long as indoxyl-sulphuric acid is combined with a base, as it is in urine, it is stable and resists oxidation; hence the coloration of urine by indigo-blue, from spontaneous oxidation of indican, is extremely rare, although that substance may be abundantly present. The oxidation cannot occur unless the acid has been previously set free, which only very exceptionally takes place from natural causes, and then it is probably due to decomposition of indoxyl-glycuronic salts, which are much less stable than the indoxyl-sulphates. Wolf¹ records the case of a patient with ileus and peritonitis, due to perforation, who passed intensely green urine of strongly acid reaction which deposited enormous quantities of indigo on standing awhile. McPhedran and Goldie² report the case of a man aged twenty-four who passed bluish-green urine containing particles of blue pigment. The residue left after extraction with chloroform, when heated, sublimed with a tinted vapour and deposited crystals of indigo-blue.

The procedure by which the presence of indican is detected in urine, and its amount estimated, consists, first, in the liberation of the acid from its base, and, secondly, in oxidation of the indoxyl-sulphuric acid or urinary indican, by which a coloured product is obtained. The pigment thus produced may be either indigo-blue, or its isomer indigo-red; when indigo-red is formed, it is accompanied by a certain amount of indigo-blue.

If a large test-tube is one-third filled with the urine, to which an equal volume of strong hydrochloric acid is added, together with a few cubic centimetres of chloroform, and then an oxidising agent, such as a minute crystal of potassium chlorate, a drop or two of a solution of bleaching powder (Jaffe), or a little peroxide of hydrogen, and, after closing the tube with the thumb, its contents are gently agitated by inversion, the urine deepens in colour, and the chloroform is tinted blue or reddish-violet. Two precautions must be observed: (a) Excess of the oxidising agent is to be avoided, else the colour will be destroyed;

¹ *Dissert.* Leyden, 1887.

² *Trans. Assoc. American Physicians*, 1901.

if potassium chlorate be used, the oxidising process takes place slowly and is consequently more under control than is the case with the bleaching powder, which acts instantaneously. An extraordinarily minute fragment of potassium chlorate suffices to initiate the oxidising process, and if time is allowed no more need be added. If bleaching powder is used the solution should only be added drop by drop, the tube being inverted between each addition; when the maximum coloration is reached, further additions diminish and, if continued, entirely destroy the colour. (b) The tube must not be vigorously shaken, lest the chloroform become emulsified with the urine, from which it will separate with difficulty, if at all. This may be prevented by Obermayer's method of precipitating the urine with about one-fifth its volume of a 20 per cent. solution of lead acetate, and filtering, before adding the hydrochloric acid; by this process the urochrome and other substances which promote emulsification are removed.

Indigo-blue.—When the chloroform extract is blue in colour it will be due to the presence of indigo-blue; this may be verified by the spectroscope, which shows an ill-defined, broadish band between C and D (Fig. 10), nearest to the latter. No other natural urinary

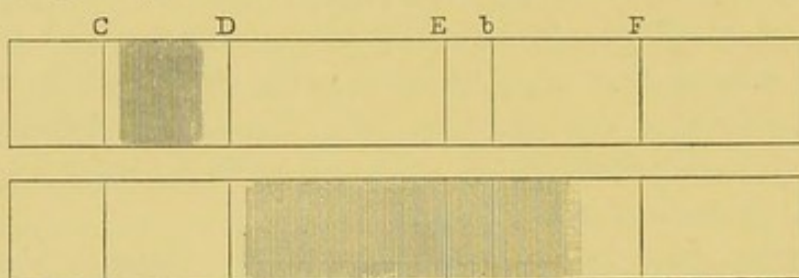


FIG. 10. Absorption spectrum of indigo-blue (upper), and of indigo-red (lower).

pigment is blue in colour; the blue or bluish-green colour of urine, which is due to the ingestion of methylene-blue (*q.v.*), has a different tint, and the urine is usually thus coloured when voided; its spectrum is also different.

Urobilin may be separated from a chloroform extract of indigo-blue or red by shaking it with a dilute solution of sodium bicarbonate, or of ammonia, into which the urobilin passes. Neither indigo-blue nor indigo-red is extracted by alkaline solutions, nor is either of them decolorised. [*Cf.* Urorosein.]

Indigo-red.—Occasionally the chloroform extract obtained is pink or bluish-red in colour, due either to the presence of indigo-red, of skatol-red, or of urorosein; if due to indigo-red (as contrasted with indigo-blue) it probably depends upon the rate of oxidation, which to some extent is a question of temperature. Maillard¹ states that slow

¹ *Compt. rendus*, 1901.

oxidation of indoxyl combinations yields indigo-red; quick oxidation yields indigo-blue. Bouma¹ treated two equal portions of the same urine in the usual way: (a) at the ordinary temperature of the room; and (b) at 45° C. (113° F.). The chloroform extract from (a) was red; that from (b) was violet. After evaporation, the respective deposits yielded (a) barely a trace, whilst (b) afforded a fair amount of indigo-blue. On the other hand, Rosin² states that indoxyl compounds yield more indigo-red if oxidation takes place at an elevated temperature, and more indigo-blue if in the cold.

*Bouma's Test*³ for Indigo.—Equal volumes of urine and of a reagent prepared by dissolving 2 mgrms. of isatin in 100 c.c. of hydrochloric acid are boiled in a test-tube; the result is that any indoxyl compounds in the urine are converted into indigo-red. When cold, a few cubic centimetres of chloroform are added, and the tube is gently shaken until the colouring matter is dissolved by the chloroform. Urine that is poor in indican yields a rose-red extract; if more indican is present the tint is purple-red; if the urine is rich in indican the extract acquires a dark wine-red.

In solutions of moderate concentration, indigo-red yields a diffuse band which extends from D nearly to F (Fig. 10). The means by which indigo-red may be distinguished from the other red pigments will be discussed in the section on urochrome.

Estimation of indigo-blue.—This may be made by Obermayer's method.⁴ The urine is first precipitated by lead acetate, as previously described; it is then filtered and 50 c.c. of the filtrate are poured into a stoppered separating funnel with an equal volume of a reagent consisting of 0.2 gm. of ferric chloride dissolved in 100 c.c. of strong hydrochloric acid. The mixture is allowed to stand for a quarter of an hour, when 25 c.c. of chloroform are added, and the funnel is shaken so as to dissolve in the chloroform as much as possible of the indigo-blue that has been formed. The chloroform is then run off into an evaporating basin, and is replaced by 10 c.c. more, which is shaken as before and then allowed to flow into the same basin. The extraction is continued with successive portions of chloroform as long as it acquires a blue tint. The united extracts are evaporated down, and the residue is mixed with 50 c.c. of 45 per cent. alcohol, and is warmed for ten minutes over a water-bath, so as to remove any foreign colouring matter. The alcohol, with the colouring matter which it has dissolved, is poured off, and the indigo-blue which remains in the basin is dried on the water-bath; it is then dissolved in 5 c.c. of concentrated sulphuric acid, the tint of the

¹ *Zeitschr. f. physiol. Chem.*, 1900.

² *Virchow's Arch.*, 1891.

³ *Zeitschr. f. physiol. Chem.*, 1902.

⁴ *Wiener klin. Rundschau*, 1898.

solution being violet-blue; if this coloration is not obtained, a little more acid must be added. The solution is gently heated on the water-bath for a quarter of an hour, and when cold is diluted with twice its volume of water, and then made up to 50 c.c. with 33 per cent. sulphuric acid. Of this 15 c.c., heated from 50 to 80° C., are titrated with a solution of potassium permanganate 0.0256 gm. to the litre, which is added at first in quantities of 0.5 c.c. and later only by drops. The end-reaction is reached when the original greenish liquid turns brown. One cubic centimetre of the permanganate solution corresponds to 0.00005 gm. of indigo-blue.

In order to remove all colouring matter except indigo-blue, Wang¹ recommends that the residue, after evaporation of the chloroform, should be washed with a mixture of equal parts of ether, alcohol, and water. He also advises that, after the hydrochloric acid and ferric chloride are added to the urine, the mixture should be shaken with the chloroform at once instead of waiting a quarter of an hour, as the delay leads to loss of indigo. Bouma² states that the estimation is from 20 to 30 per cent. too low if Wang's method of purifying be adopted, and prefers to wash the chloroform extract with distilled water, and, after evaporation of the extract, to heat the residue on the water-bath at the boiling-point for half an hour. He also recommends immediate extraction with the chloroform, after the addition of the oxidising agent, and again in half an hour.

SKATOXYL COMBINATIONS.

Skatoxyl, or methyl-indol, is an oxidised product of skatol, which itself is a more advanced product of intestinal putrefaction than its congener indol. Like indoxyl, skatoxyl conjugates with sulphuric acid, and, according to Brieger,³ when present in urine, it appears for the most part as potassium skatoxyl-sulphate; it may also be present as a salt of skatoxyl-glycuronic acid. Salkowski⁴ described another conjugated product of skatol—skatol-carbonic acid—which he states is present in minute traces in human urine. Blumenthal⁵ found skatol-carbonic acid in the urine from cases of pneumonia, phthisis, cancer of the stomach and of the intestines, and other diseases. Most of the skatol that is formed in the intestinal canal is excreted with the fæces; under normal conditions only a small

¹ *Zeitschr. f. physiol. Chem.*, 1899.

² *Ibid.*, 1899 and 1903.

³ *Berichte d. deutsche chem. Gesellsch.*, 1879.

⁴ *Zeitschr. f. physiol. Chem.*, 1885.

⁵ *Deutsche Klinik*, 1901.

amount is absorbed, and consequently its oxidation product appears less constantly and in smaller amount in the urine than is the case with the oxidation product of indol. The amount of skatol in the intestines is increased by the same conditions which cause an excess of indol. Potassium skatoxyl-sulphate is a crystalline substance which is soluble in water, but is only slightly soluble in alcohol. Stokvis¹ believes that the chromogen of skatol-red is not an ether-sulphate, as, when heated with acids, it yields neither sulphuric acid nor any reducing substance.

Detection.—Equal volumes of the urine and of hydrochloric acid are put into a test-tube along with a little amyl alcohol and a minute fragment of potassium chlorate, or a drop or two of a solution of bleaching-powder, and the tube is repeatedly inverted; the urine acquires a reddish colour and the amyl alcohol a rosy-red. Rössler,² following Nencki and Sieber's method for developing urorosein, recommends that, after precipitation with lead acetate, 10 c.c. of the urine with an equal volume of hydrochloric acid should be mixed in a test-tube, and, after standing for five minutes, should be extracted with 5 c.c. of amyl alcohol. The extract thus obtained will be brownish-red in colour. Rössler states that skatol-red gives an absorption band between C and D. If the extract which contains skatol-red is shaken with an alkali, the extract loses colour and becomes pale yellow.

UROROSEIN.

This substance was discovered in urine by Nencki and Sieber.³ It is present as a chromogen, the rosy-red pigment being only revealed after the addition of an oxidising agent. Its development often accompanies the use of nitric acid as a test for albumin; the red or reddish-brown tint imparted to the layer of urine which rests on the acid is chiefly due to urorosein; when much of the pigment is present a bright rosy tint spreads upwards through the column of urine. The chromogen of urorosein is probably present in very small amount in normal urine. Urorosein possesses the following properties: it is soluble in water, amyl alcohol, and acidulated ethyl alcohol; less so in neutral ethyl alcohol, and very slightly so in acetic ether. It is insoluble in ether, chloroform and benzene. It can only be extracted from urine by means of amyl alcohol. Nencki showed that the pigment dyes sheep's wool, and Rosin⁴ points out that

¹ *Handl. Nederland. Natuur-en Geneesk. Congres*, 1901.

² *Centralbl. f. innere Med.*, 1901. ³ *Journ. f. prakt. Chem.*, 1882.

⁴ *Virchow's Arch.*, 1891.

it has an affinity for the fibre of filter-paper, so that a solution of uro-rosein repeatedly passed through the same filter colours it red. The pigment acts as an acid and forms colourless salts with the alkalis, which are soluble in water, alcohol, amyl alcohol, chloroform, and ether; from solutions of its salts the colour of the pigment is restored by mineral acids, but not by organic acids. Urorosein is unstable, and quickly loses its colour. The spectrum of urorosein is very characteristic: it consists of a band in the green, nearly midway between D and E, slightly nearer to D (Fig. 11).

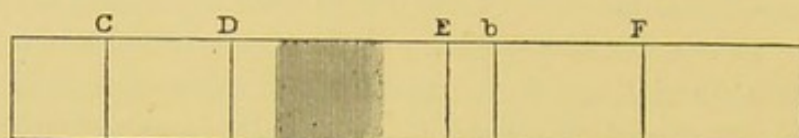


FIG. 11. Absorption spectrum of urorosein.

The amount of urorosein in urine is increased by vegetable diet, hence the urine of herbivorous animals, especially oxen, contains large quantities. In human beings, various pathological conditions tend to produce an excess of the chromogen: in advanced phthisis and other forms of tuberculous disease, in malignant disease of the abdominal organs, gastric ulcer, dilatation of the stomach, perityphlitis, typhoid fever, pernicious anæmia, in some cases of chlorosis and in diabetes, a more or less pronounced increase is met with.

The presence in urine of a red pigment, which is developed by oxidising agents, and is insoluble in chloroform, but is freely soluble in amyl alcohol, and which is rendered colourless by shaking its solution with an alkali, regaining its colour on acidulation, is far from infrequent. Such a pigment (or, rather, its chromogen) may be present for prolonged periods in the urine of individuals that are apparently in good health. Systematic examinations, extending over many years, of the urines of a large number of persons, some in perfect health and others with trifling ailments not affecting the digestive tract, have shown how greatly the "personal equation" has to be taken into account, and have convinced me of the futility of accepting any excess of pigment that is not very considerable (unless the constant of the individual is known) as a guide to diagnosis.

Detection and Separation.—Nencki and Sieber showed that when 10 per cent. of hydrochloric acid is mixed with the urine the pigment of urorosein is slowly developed, oxidation being effected by the atmospheric oxygen; if the mixture of acid and urine be warmed, oxidation takes place more quickly. Excellent results are obtained by Obermayer's method of oxidising urinary indican (*q.v.*), by treating the urine with lead acetate, and the filtrate therefrom with an

equal volume of hydrochloric acid containing 0.2 per cent. of ferric chloride; the pigment, which is slowly produced, is extracted with amyl alcohol. Or, after treatment with lead acetate and the addition of hydrochloric acid, oxidation may be effected by means of a minute crystal of potassium chlorate. When necessary, Rosin's¹ method of purification may be adopted; this consists in shaking the alcoholic extract with a dilute solution of potash, or of ammonia, which causes it to lose colour owing to the combination of the pigment with the alkali; at the same time the aqueous alkaline solution becomes tinted by the extraneous colouring matter that it dissolves out of the alcohol. If the alcohol is now separated and acidulated by shaking with a little hydrochloric acid it re-acquires the rosy tint, more or less freed from impurities. Rosin recommends a simple method of extraction: after the pigment has been developed the urine is passed repeatedly through a few filter-papers, and, after washing them with ether and chloroform, the pigment is dissolved by digesting the papers in ethyl alcohol.

When some urines are treated with hydrochloric acid and an oxidising agent they acquire a bright pink colour, and yet, if chloroform be used as the solvent, it is tinted blue, the colour of the urine being practically unaltered, or perhaps it becomes a brighter red. If the urine is now decanted from the chloroform and is shaken with amyl alcohol its colour is transferred to the solvent, which becomes rose-coloured. This is due to the associated presence in the urine of indigo-blue and urorosein; therefore, in clinical testing for urorosein, it is well first to shake with chloroform in order to remove any indigo-blue that is present, otherwise both pigments will pass into the amyl alcohol and thus embarrass the spectroscopic examination.

It is to be observed that all reddish coloration which is produced in urine by oxidising agents, and which cannot be extracted by chloroform, but can be easily extracted by amyl alcohol, is by no means necessarily or entirely due to urorosein; much is often due to other pigments, the nature of which is unknown. Any urine, on prolonged boiling with hydrochloric acid, darkens to a reddish-brown, or until it is almost black; some of this coloration may be due to the presence of unknown chromogens, but it chiefly results from dehydration of the carbohydrate substances which are present in urine and which have no claim to be regarded as chromogens; consequently coloration thus produced has no clinical significance. These coloured products are soluble in ethyl and amyl alcohols, but not in ether, acetic ether, nor chloroform. They show no absorption bands.

Isolation of the Red Pigments.—As the same means are used to

¹ *Loc. cit.*

develop indigo-red, skatol-red, and urorosein from their respective chromogens, it follows that if these chromogens are all present in the same urine some mode of separation must be adopted in order to enable the pigments to be severally identified.

The separation of the chromogens of indigo-red and skatol-red may be effectively accomplished by Stokvis's¹ method, which consists, first, in saturating the urine with ammonium sulphate; this removes uroerythrin, urobilin, bile-pigment, and hæmatoporphyrin. After filtration the filtrate is concentrated on the water-bath; when cold the liquid is removed from the excess of ammonium sulphate, is acidulated with a few drops of acetic acid, and shaken with an equal volume of acetic ether, to which both of the chromogens are transferred. After separation the ether extract is repeatedly well shaken with distilled water, which, being acidulated by the acid ether, takes up the indigo chromogen. The ether is again separated and is shaken up with a moderately strong solution of potash, which dissolves out the skatol chromogen and acquires a yellow tint. The acid aqueous solution is oxidised, as before described, with hydrochloric acid and bleaching-powder, and the resulting indigo-red is extracted with chloroform. The alkaline aqueous solution is similarly oxidised, and the skatol-red is extracted with amyl alcohol.

The separation of skatol-red from urorosein is a much more difficult matter. Skatol-red and urorosein are extracted from urine by amyl alcohol, and both combine with alkalies and form colourless products (salts?), which yield the original colour on acidulation. The chromogen of urorosein is not an ether sulphate, neither (according to Stokvis) is that of skatol-red. The spectrum of urorosein consists of a band between D and E; that of skatol-red is stated by Rössler to consist of a band between C and D, whilst Stokvis states that it gives two bands between D and E. The differentiation between skatol-red and urorosein is obscure, and awaits further elucidation. Garrod and Hopkins's² observation that the chromogen of urorosein is precipitated by saturation with ammonium sulphate which, according to Stokvis, leaves the skatol chromogen in solution, is suggestive, but the precipitation is only partial. At present the only trustworthy method of identifying skatol-red is that recommended by Huppert,³ viz., to obtain the pigment in the solid form and then to ascertain whether it yields skatol when heated with zinc dust. It is more than probable that the rosy tint developed in urine by oxidising agents, which is

¹ *Handl. Nederland. Natuur-en Geneesk. Congres*, 1901.

² *Journ. of Physiol.*, 1896.

³ Neubauer and Vogel, 1898.

assumed to be due either to skatol-red or to urorosein, is almost invariably due to urorosein.

ALKAPTONURIA.

In this rare condition the urine is of a natural colour when voided, but, subsequently, it becomes brown and eventually black on exposure to air; the change in colour is furthered by alkalisation of the urine. With the aid of heat alkapton urine reduces copper salts in alkaline solution, but not those of bismuth; it reduces a solution of ammonio-nitrate of silver in the cold. Alkapton urine does not undergo saccharine fermentation, nor does it rotate the plane of polarised light. Alkaptonuria was first described in 1859 by Boedecker,¹ who attributed its properties to a substance which he named alkapton, from alkali and *καπτείν*; since then the abnormality has been investigated by many observers, and the conclusion arrived at is that the coloration is due to an aromatic carboxylic acid. Kirk² found uroleucic acid which, according to Huppert,³ is probably dioxybenzene-lactic acid; this is the only case in which uroleucic acid has been found. Wolkow and Baumann⁴ found that homogentisic acid, which they regard as hydroquinone-acetic acid, is always present, and they believe it to be the sole reducing agent in alkaptonuria. Garrod⁵ considers that the presence of homogentisic acid is the essential feature of alkaptonuria, and that uroleucic acid, when present, is of the nature of a by-product. This he has confirmed by examining, in 1902, the urine from Kirk's cases, which he finds no longer contains uroleucic acid.

Alkaptonuria is commoner in males than in females; it usually exists from birth or early childhood, and persists throughout life. Several cases are recorded in which the urine manifested all the characteristics of alkaptonuria on the day after the child was born; that is, as soon as any proteids reached the intestines, allowance being made for the oxidising power of the tissues to destroy a certain amount of the homogentisic acid that is first formed. The first case of alkaptonuria in which homogentisic acid was found in the urine was that of a man sixty-eight years of age, who had been alkaptonuric all his life (Baumann and Kraske⁶); the sister of this man was sixty years of age when her case was investigated by Embden,⁷ and she also had been alkaptonuric from birth. It is stated that, occasionally, alkaptonuria develops in later life, sometimes after an

¹ *Zeitschr. f. rat. Med.*, 1859.

² *Brit. Med. Journ.*, 1886.

³ *Zeitschr. f. physiol. Chem.*, 1897.

⁴ *Ibid.*, 1891.

⁵ *Trans. Med. Chirurg. Soc.*, 1899 and 1902.

⁶ *Münchener med. Wochenschr.*, 1891. ⁷ *Zeitschr. f. physiol. Chem.*, 1893.

illness, and also that it may be intermittent; it is almost always congenital and constant, and so long as the daily excretion of homogentisic acid is not excessive, it does not impair the health. It runs in families, though not from parent to child, but it affects several children of the same parents. Garrod¹ quotes evidence to show that the children of first cousins are specially liable to alkaptonuria. When the early commencement and the prolonged duration of alkaptonuria are taken into consideration, it seems highly probable that the condition is due to an inherent metabolic idiosyncrasy pertaining to the individual. Abderhalden and Falta,² from evidence obtained experimentally, infer that the fundamental disturbance which gives rise to alkaptonuria is not to be sought for in the intestinal canal (as held by Baumann and others), but that it is a specific derangement of proteid metabolism, which is comparable with the anomalous metabolism that causes cystinuria.

MELANURIA.

Melanin is another substance which occasionally appears in the urine, and, like homogentisic acid, causes it to darken on exposure to air. When voided the urine is usually of normal colour; sometimes the freshly-voided urine has a brownish-yellow or even a brownish tint. The pigmentary substance occurs in the chromogen-form as melanogen; after the pigment is developed from its chromogen it remains in solution, except on rare occasions when it takes the form of discrete granules. Oxidising agents, such as hydrochloric acid and potassium chlorate, or dilute sulphuric acid and potassium dichromate, added to urine which contains melanogen, rapidly produce the dark colour, which develops slowly by mere exposure to air. When the pigment has been produced by exposure of the urine to the air, it can be reduced by nascent hydrogen—liberated by the addition to the urine of hydrochloric acid and metallic zinc—with consequent loss of colour. Hofmeister found that sodium amalgam slowly decolorised the pigment. Pollak³ states that when the pigment has been developed from its chromogen by chemical reagents, reducing agents have no effect upon it. Bromine-water, when added to melanin-urine, produces a yellow precipitate, which gradually blackens. Sodium nitroprusside with caustic potash produce a purple-red colour, which, on acidulation, changes to deep blue; this test is not reliable, as other

¹ *The Medico-Chirurg. Trans.*, 1899, and *The Lancet*, 1902.

² *Zeitschr. f. physiol. Chem.*, 1903. ³ *Wiener med. Wochenschr.*, 1889.

substances beside melanogen, which may be present in urine, yield a similar reaction. A fairly concentrated solution of ferric chloride causes a grey coloration when added to urine that contains melanogen. (von Jaksch.) On the other hand, Helman-Lodz¹ states that the presence of true melanogen in urine is only to be accepted when ferric chloride produces a black precipitate which is soluble on the addition of sodium carbonate, and is again thrown down, by mineral acids, as a black or brownish-black powder.

Two views are held as to the derivation of melanin: one, that it is derived from blood pigment, and the other, that it is an albumin-product, not derived from the blood. Berdez and Nencki² believe it to be a condensation product of albumin, and Schmiedeberg,³ whilst stating that it has not a constant composition, regards it as a cleavage-product of albumin. Stokvis⁴ found a large excess of neutral sulphur in the urine from a case of melanuria; on some days the neutral sulphur exceeded the total amount of mineral and ether sulphates; he also points out that the reactions given by melanin-urine embarrass the interpretation of the tests for indican and acetone. The enzyme *tyrosinase* (cf. homogentisic acid) has been found in animal, as well as in vegetable juices, and it appears to possess the power of converting tyrosin into melanin; the melanin produced by it has the same elementary composition as that assigned to melanin by Hofmeister, and on being fused with potash it yields the odour of indol. (v. Fürth and Schneider.⁵)

The occurrence of melanuria affords no proof of the presence of pigmented growths: patients with wasting diseases may have melanuria, and it may be absent in those who are the subjects of melanotic tumours. The occurrence of melanuria, however, demands careful investigation of the patient, as regards the possibility of such a growth being present. (v. Jaksch.)

BLOOD-COLOURING MATTER IN URINE.

The colouring matter of blood may be present in urine either as oxyhæmoglobin, as methæmoglobin, or as hæmatin; it may be retained in the red corpuscles, constituting *hæmaturia*; or it may be free, in solution in the urine, constituting *hæmoglobinuria*, the former being much more common than the latter. When hæmatin is present the condition is known as *hæmatinuria*.

¹ *Centralbl. f. innere Med.*, 1902.

² *Arch. f. Path.*, 1886.

³ *Ibid.*, 1897.

⁴ *Nederlandsch Tijdschr. v. Geneesk.*, 1899.

⁵ *Beiträge z. chem. Physiol. u. Pathol.*, 1901.

HÆMATURIA.

The appearance of urine that contains blood varies considerably with the amount of blood that is present. When urine contains a large amount of blood the appearance is that of blood mixed with water, and the deposit which falls on standing is voluminous and red in colour. Copious hæmorrhage is often of vesical origin, from a neoplasm or from tubercle; hæmorrhage due to stone is not so abundant; in either case clots, sometimes of considerable size, may be present. During micturition, a distinctive indication of the vesical origin of the blood is sometimes afforded by an excess of blood in the last portion of urine voided; not unfrequently the earlier portion is almost, or quite, free from blood, which only appears in the last ounce or two. If the prostate or the urethra is the source of blood, some will probably trickle down the urethra and stain the linen in the intervals between micturition, and in the act of micturition a small clot may precede the flow of urine. Copious hæmorrhage from the kidneys suggests trauma, cancer, or the presence of animal parasites such as *Distoma hæmatobium* and *Filaria sanguinis hominis*, when clots may be present in the urine, as they also may when the renal hæmorrhage is due to calculus; more restricted hæmorrhage occurs after toxic doses of turpentine, cantharides, and other poisons. In inflammatory conditions of the kidneys, attended by hæmaturia, the hæmorrhage is usually most copious and persistent in the acute nephritis due to "taking cold." The blood derived from an inflamed kidney is gradually exuded and is intimately mixed with the urine; it tends to undergo changes whilst within the urinary passages, which are especially obvious when the amount of blood is small. Under these conditions the urine looks dusky or smoky, and to the untrained eye does not suggest the presence of blood; the deposit, dirty-brown in colour, is not very dense, and it probably contains casts—occasionally blood-casts—but no clots. Certain general diseases, such as purpura hæmorrhagica, scurvy, and some fevers, and also extensive burns, may give rise to hæmaturia.

HÆMOGLOBINURIA.

When hæmoglobin, freed from its corpuscles, is dissolved in the urine, the appearance differs from that due to hæmaturia; the abundant presence of hæmoglobin no longer causes the urine to look like blood and water, but rather to resemble some black

liquid, like porter; it is only when viewed as a thin layer, or when diluted with water, that the red tint is revealed. The urine deposits a dark-brown or blackish sediment, consisting of granular matter without any red corpuscles; or possibly one or two may be found. When some of the urine is boiled in a test-tube the hæmoglobin coagulates and appears as a diffuse brown clot, which rises to the upper part of the urine.

Hæmoglobinuria occurs as the consequence of destruction of a number of the red corpuscles within the blood vessels; hæmoglobinæmia results, and then the free hæmoglobin is removed from the blood plasma by the kidneys. Disintegration of red corpuscles may be caused by toxins which are exceptionally formed in certain pathological conditions, in scarlet, black water, and other fevers, for example; by blood poisons from without, such as arsenetted hydrogen, potassium chlorate, pyrogallol; and by cold acting on unstable corpuscles, as in that rare disease paroxysmal hæmoglobinuria.

In hæmatinuria the urine has a brown tint and does not necessarily coagulate with heat; it deposits a dark-brown, often rather compact sediment. Along with hæmatin, hæmoglobin or methæmoglobin may be present. Hæmatinuria has been observed in cases of poisoning by sulphuric acid and potassium chlorate.

DETECTION OF BLOOD IN URINE.

The guaiacum test is thus performed: To a little of the urine in a test-tube a few drops of fresh tincture of guaiacum and some ozonic ether are added; when the tube is gently shaken a blue coloration appears if blood is present. This test is very unreliable; many substances which may be accidentally present in urine give the same reaction.

If a little of the deposit from urine containing blood, free hæmoglobin, or hæmatin is allowed to dry on a microscope slide and is then well mixed with a minute granule of sodium chloride and a couple of drops of glacial acetic acid, and after being covered with a thin glass-cover is heated in the flame of a spirit-lamp until it begins to boil; on cooling, small crystals of hæmin may be seen under the microscope. These crystals have a reddish-brown colour, and appear as elongated rhombic plates with bevelled ends, frequently arranged in crosses or groups. When very small they are bi-convex, like minute uric acid whetstone-crystals.

A little of the urine, diluted if necessary, when examined with the spectroscope—a small direct-vision, pocket instrument is the most convenient—shows either the two bands of oxyhæmoglobin alone,

between D and E; or, with the addition of a narrow band in the red between C and D, indicating the presence of methæmoglobin. Both these spectra are changed to the single broad band of reduced hæmoglobin on the addition to the urine of a few drops of ammonium sulphide. When the hæmoglobin spectrum is difficult to obtain, a little strong solution of potassium hydrate should be added to the urine; this converts the hæmoglobin into alkaline hæmatin, and by the addition of a drop or two of ammonium sulphide the hæmatin is reduced to hæmochromogen which yields the most distinct of the blood-spectra—two bands rather nearer the violet end of the spectrum than those of oxy-hæmoglobin. A spectrum with a narrow band in the red, which closely resembles that of methæmoglobin, may be due to the presence of acid hæmatin. The distinction between these substances is made by feebly alkalising the urine with ammonia, filtering, and adding a few drops of ammonium sulphide: the methæmoglobin spectrum changes to that of reduced hæmoglobin, whilst the hæmatin gives place to hæmochromogen.

When the amount of blood in the urine is too small to yield a direct spectroscopic reaction, Sonnenschein's¹ method may be used with advantage. Some of the urine, freely acidulated with acetic acid, is precipitated with a strong solution of sodium tungstate; the precipitate is collected on a filter and washed to clear it from phosphates, and is then dissolved in dilute ammonia. The solution yields the spectrum of methæmoglobin; mere traces of blood can be detected by this method.

In clinical work, the detection of a trace of blood in the urine is usually and most readily accomplished by means of a microscopical examination of the deposit which is formed on standing, or after using the centrifuge; the discovery of red blood-corpuscles (*q.v.*) proves the presence of blood. In hæmoglobinuria or in hæmatinuria blood-corpuscles will probably be absent; should the amount of hæmoglobin or of hæmatin be too small for direct spectroscopic examination the former may be precipitated with sodium tungstate, as just described, and the latter may be extracted from the urine with ether, in which its spectrum will probably be visible; by agitating the ethereal extract with weak ammonia-water the hæmatin is transferred to the aqueous solution, and may be reduced with ammonium sulphide, and so made to yield the bands of hæmochromogen.

¹ *Vierteljahrsschr. f. ger. Med.*, 1873.

BILE-PIGMENTS.

Bile-pigments are not present in normal urine ; when they are present it is in consequence of obstruction of the bile-ducts, which may be due to a number of causes. When this occurs, the stoppage of the natural passage of the bile into the duodenum leads to its absorption and transmission to the blood, whence it is excreted by the kidneys. The bile-pigments are derived from the hæmatin-component of hæmoglobin, the iron of which is retained by the liver-cells.

BILIRUBIN. ($C_{32}H_{36}N_4O_6$.)

Bilirubin is the least oxidised of the biliary pigments, which are chiefly represented by it in the urine. It is insoluble in water, is slightly soluble in alcohol, and is freely soluble in chloroform. Its solutions, which are yellow or brownish-red in colour, show no bands with the spectroscope. By oxidising agents bilirubin is converted into biliverdin, and then into bilicyanin, and finally into choletelin. By reducing agents (nascent hydrogen) it is converted into hydrobilirubin, which is very closely allied to, if not actually the same substance as, urobilin (*q.v.*) ; this substance constitutes the link between abnormal and normal urinary pigments derived from blood : bilirubin is an abnormal urinary pigment, whilst urobilin is a normal urinary pigment.

BILIVERDIN. ($C_{32}H_{36}N_4O_8$.)

Biliverdin may be regarded as oxidised bilirubin. It is insoluble in water, ether, and chloroform ; and is soluble in alcohol and in acetic and hydrochloric acids. When submitted to the action of oxidising agents it gives the same sequence of products as bilirubin, and, like it, yields no absorption bands.

BILICYANIN.

Bilicyanin, or cholecyanin, is a further oxidation product, which is only obtainable artificially by means of the action of strong oxidising agents, such as nitric acid, on either bilirubin or biliverdin. It is chiefly of interest to the physician as being the substance to which the blue zone seen in Gmelin's test is due. In contrast to the two preceding pigments bilicyanin gives a distinct spectrum, which, in acid solution of medium concentration, shows two bands symmetrically placed, one on each side of the D line. A third band

between E and F is sometimes seen, which probably belongs to choletelin, as it only occurs at a more advanced stage of oxidation.

The last oxidation product with nitric acid is **choletelin**, which furnishes the yellow of Gmelin's test; its spectrum is limited to the band between E and F, just mentioned. Other substances, such as **biliprasin** and **bilifuscin**, are described amongst the bile-pigments, but they are not of practical moment.

Urine that contains bile may vary in colour from the ordinary yellow of normal urine through deep yellow, reddish-brown, to absolute black when viewed in bulk. Occasionally, owing to the presence of an oxidising ferment, or from some other cause, bile-stained urine is green, or has a greenish hue, which indicates that the whole or a part of the pigment occurs as biliverdin, the usual form in urine being bilirubin. Ordinary dark, bile-stained urine often shows a green shimmer on its surface where it is exposed to the air, due to absorption of oxygen. The froth of bile-stained urine is usually yellow, but it may be greenish in colour. Caution should be exercised in the interpretation of the colour of the urine or of its froth; urine which contains much urobilin, and no bile-pigment, often so closely resembles the urine of jaundice as to render inspection with the naked eye a very uncertain means of differentiation. The froth produced by shaking bile-stained urine is more lasting than that of ordinary urine; this is due to the presence of taurochol- and probably nucleo-albumin derived from the bile-passages. The addition of nitric acid to such urine gives rise to a cloud which is another manifestation of the same substance; on gently warming the urine the cloud disappears. Gilbert and Lereboullet¹ direct attention to the fact that the skin may have an icteric tinge, and that the blood may contain bile-pigment, and yet barely a trace, if any, may be present in the urine.

TESTS FOR BILE-PIGMENTS IN URINE.

Gmelin's Nitric Acid Test.—If a little nitric acid, that by exposure to sunlight has turned yellow in colour and gives off fumes of nitrous acid, is put into a test-tube, and some bile-containing urine is gently poured over it, the layer of urine in contact with the acid becomes reddish-yellow, due to the formation of choletelin, the most highly oxidised of the bilirubin products; further away from the acid the colour is redder and more purple, and it then changes to blue, which is due to bilicyanin; and lastly to green from the forma-

¹ *Compt. rend. Soc. Biolog.*, 1901.

tion of biliverdin, the least oxidised of the series. Of these colours the green is the only one which demonstrates the presence of bile-pigment; all the others may be due to oxidation of hæmatoporphyrin and various other chromogens; therefore, unless the green tint is produced, the reaction is to be regarded as negative. If the urine is dark-coloured (as it may be from urobilin without much bile-pigment) it should be diluted with water to S.G. 1005 (Zeehuisen¹) before being tested, and if albumin is present it should be removed by boiling.

The *iodine test*, first described by Trousseau and Dumontpallier,² though usually ascribed to Maréchal,³ is best applied as modified by Rosin.⁴ A little 1 per cent. alcoholic solution of iodine (which is represented by about equal parts of the B.P. tincture of iodine and rectified spirit) is gently floated on the surface of some of the urine in a test-tube: at the plane of contact a green disc forms; if the tube is agitated the whole of the urine becomes green. Should the urine be alkaline, it must be previously rendered slightly acid with a drop or two of acetic acid. Zeehuisen prefers a still weaker alcoholic solution of iodine—1 : 500 to 1 : 3000.

The green tint is probably due to biliverdin, although Maly⁵ states that the green which is produced by the action of bromine with bilirubin is a substitution-product and not the result of oxidation; still, when the bromine is expelled from this substitution-product—tribromo-bilirubin—biliverdin remains. Zeehuisen⁶ finds that a green coloration is produced by iodine with some normal urines; this has also been observed by the author. The oxidising power of iodine being lower than that of nitric acid, the only oxidation-product produced is biliverdin; the consequent absence of other colours renders the test more distinctive and delicate than Gmelin's test.

Various methods have been devised for the identification of bile-pigments by separating them from the urine; as regards most of these methods, any assumed advantage in point of delicacy does not compensate for the time required to perform the tests. Probably the best and most delicate is Salkowski's⁷ modification of Huppert's process, which may be advantageously resorted to when the amount of bile-pigment is exceptionally small.

Huppert-Salkowski method of separating bile-pigment. Into a little of the urine, slightly alkalisied with sodium carbonate, a solution of calcium chloride is dropped as long as a precipitate

¹ *Zeitschr. f. klin. Med.*, 1895.

² *L'Union Méd.*, 1863.

³ *Zeitschr. f. analyt. Chem.*, 1869.

⁴ *Berliner klin. Wochenschr.*, 1893.

⁵ *Wiener Akad. Sitzungsberichte*, 1875.

⁶ *Zeitschr. f. klin. Med.*, 1895.

⁷ *Lehre vom Harn*, 1882.

forms; the precipitate is filtered off, is washed, and is then dissolved, with the aid of a little hydrochloric acid, in about 10 c.c. of alcohol. The clear solution when boiled becomes green or blue. After the solution is quite cold, the addition of nitric acid causes it to turn blue, violet, and red.

BILE ACIDS.

Glycocholic acid ($C_{26}H_{43}NO_6$) is a monobasic acid which crystallises in delicate needles that are soluble in alcohol, but are only slightly soluble in water; its salts are soluble in both.

Taurocholic acid ($C_{26}H_{45}NSO_7$) is also monobasic and crystallises in needle-shaped crystals; it is freely soluble both in water and in alcohol, as are also its salts. Both these acids occur in human bile in combination with sodium, forming the bile salts which, as well as the free acids, are optically active, rotating the plane of polarised light to the right. The bile acids are not present in normal urine, but they appear, sometimes in considerable amount, in icteric urine. It is fortunate that the bile acids are not of much clinical importance, for they cannot be satisfactorily identified by chemical tests applied directly to the urine; they need to be isolated, which involves a troublesome process, and an expenditure of time vastly in excess of any practical information that can be obtained.

If isolation be required, Hoppe-Seyler's method may be adopted: The urine is precipitated with lead acetate and a little ammonia; the precipitate, after being washed and dried, is extracted at a gentle heat with absolute alcohol. After the addition of a few drops of a solution of soda the alcoholic extract is evaporated to dryness, and the residue is boiled with absolute alcohol which, by evaporation, is reduced to a small volume; after cooling, the bile-acid salts are precipitated by the addition of a large volume of ether. At first the precipitate is amorphous; but, after standing a considerable time, it takes the form of fine crystalline needles.

TESTS FOR BILE ACIDS IN URINE.

Pettenkofer's test is the one commonly described; but when applied to a complex liquid like urine, especially in the presence of bile-pigments, it is absolutely unreliable. It is performed by mixing a few drops of the urine, on a white porcelain surface, with a drop of sulphuric acid, allowing as little heat to develop as possible; a drop of a 10 per cent. solution of cane-sugar is added; a positive reaction is shown by the appearance of a red and then a reddish-purple

colour. Various modifications of this test have been proposed, but none that can be successfully applied directly to urine. The colour reaction, when obtained, should be identified by the spectroscope, which shows a band between D and E, and another at F.

*Hay's Test.*¹—This is not a chemical test, but is founded on the fact that the presence of bile salts in a liquid greatly reduces its surface-tension. After ascertaining the occurrence of this phenomenon, Hay proposed a very simple method of demonstrating the presence of bile salts by sprinkling sublimed or precipitated sulphur on the liquid that contains them. If sprinkled on water the sulphur will remain on the surface for an indefinite time; but if bile acids are present it sinks, sooner or later, in accordance with their percentage. If bile acids are present in from 1 : 5000 to 1 : 10,000 the sulphur at once begins to sink and is all precipitated in two or three minutes; even in a dilution of 1 : 120,000 precipitation occurs, though of course much more slowly. Hay states that no other substances in the body, except soaps, have the same action as the bile acids in anything like the same degree. Beddard and Pembrey² apply the test by throwing some sublimed sulphur on the urine in a wide test-tube an inch in diameter. If, at once, any begins to fall, bile salts at least 1 : 10,000 are present; if none falls the tube is gently shaken, when if some now begins to fall at least 1 : 40,000 are present, and so on for further dilutions. According to Zanfrognini and Lancellotti,³ urobilin reduces the surface-tension of liquids, and, when present in urine in great excess, may vitiate the results obtained by Hay's test.

Cluzet,⁴ acting on Hay's discovery, tests the surface-tension by means of a drop-tube which delivers 1 c.c. of distilled water at 15° C. in twenty drops. Fresh, filtered normal urine gives twenty to twenty-six drops; when the number exceeds thirty the presence of bile salts is indicated. He also uses for testing surface-tension a capillary tube three-tenths of a millimetre in diameter, which is graduated in millimetres and is furnished at its upper end with a rubber ball. The urine at 15° C. is placed in a small vessel and in it the tube up to the zero mark; the ball is then worked once or twice so as to cause some of the urine to ascend and descend the tube, when the ball is detached, and the level of the urine in the tube is read off by means of the scale. Urine containing bile salts shows a capillarity below 80 mm.; distilled water shows 114 mm. Cluzet states that the thirty drops given with the drop-tube and

¹ Landois and Stirling's *Physiology*, 1886.

² *Brit. Med. Journ.*, 1902.

³ *Soc. med. di Modena*, 1903.

⁴ *Compt. rend. Soc. Biol.*, 1901.

the 80 mm. with the capillary tube correspond to a surface-tension of fifty-five dynes to the centimetre. Meillère¹ repeats the drop-method, using the drop-counter of Duclaux with a capacity of 5 c.c. which, with urine of medium concentration and at room temperature (17.5° C.), gives 107 to 110 drops—distilled water giving 101. A solution of human bile 1 : 100 gave 128 drops; 1 : 1000 gave only 103. On the other hand, a 1 per cent. solution of sodium glycocholate gave 150 drops. From these results Meillère infers that reliable information cannot be obtained from the surface-tension method unless the bile acids are separated from the urine. This would destroy the beautiful simplicity of Hay's test, which constitutes the only method that the clinical physician can adopt in order to ascertain the presence or the absence of bile salts in urine, and for this purpose it is amply sufficient.

¹ *Compt. rend. Soc. Biol.*, 1901.

ADVENTITIOUS PIGMENTARY AND OTHER SUBSTANCES.

URINE sometimes acquires a peculiar tint from the presence of some foreign colouring matter. Amongst the **vegetable substances** which impart colour to urine are : beetroot, bilberries, blackberries, and other fruits, which yield dark-coloured juices. When rhubarb or senna is taken, especially if in repeated doses, chrysophanic acid is excreted in the urine, and usually imparts to it a distinctive yellow colour, suggestive of a small amount of bile-pigment ; this may occur after a single dose of liquorice powder. The same condition of the urine is produced, by absorption, when chrysophanic acid is applied to the skin in the form of ointment or paint. On the addition of an alkali to such urine a dull, reddish tint develops, of a different hue to any which is caused by pigments formed in the body ; on subsequent acidulation the colour disappears or changes to light yellow. Santonin imparts a yellow colour to the urine, which, although not due to chrysophanic acid, is changed to red on the addition of a little potash or soda ; the similar coloration which an alkali produces with urine from a patient taking rhubarb is distinguished by adding excess of milk of lime to some of the urine, and then filtering it : the colour due to santonin persists, whilst that due to rhubarb is carried down by the precipitate, leaving the urine colourless. The urine of a person taking santonin is coloured a bright milky yellow on the addition of calcium carbide, even a day or two after the last dose was taken. (Cronzel.¹)

Some resinous drugs, such as **copaiba**, cause the urine of patients who are taking them to develop a white cloud, which extends throughout the entire column of urine, when it is tested for albumin with nitric acid ; if the urine is heated the cloud does not disappear, but it becomes brownish-red in colour. If, in place of heating the cloudy urine, some alcohol is added, the cloud is dissolved and the urine is rendered clear ; should albumin be present, it will be indicated, according to its amount, by a disc or cloud which persists in the usual position, immediately above the stratum of acid, after the

¹ *Annal. d. Chim. analyt.*, 1902.

resinous cloud has been dissolved. On freely adding hydrochloric acid to urine from a patient who is taking copaiba the urine becomes cloudy, and the cloud shortly acquires a reddish colour; the pigment is not dissolved on shaking with chloroform. The urine from patients taking **sandal-wood oil** becomes cloudy, but does not change colour, on the addition of an acid. Such urines have a characteristic odour which recalls the odour of the drug; if not at once obvious, it may be developed by heating some of the acidulated urine, or, after filtration, the filter-paper will yield the odour.

Turpentine, when excreted in the urine, imparts to it the odour of violets, and gives a white precipitate on the addition of a mineral acid.

Anilin dyes not unfrequently give rise to peculiar colorations of urine, more especially in children, on account of the common use of some of these dyes to tint sweetmeats. One such pigment is eosin, which imparts to the urine a pinkish-red tint with a strong green fluorescence. If some of the urine thus tinted is put into a test-tube and is extracted with a little amyl alcohol the solvent becomes coloured with the dye. On spectroscopic examination of the extract of moderate strength it will be found to give a well-marked band, beginning midway between D and E, and continuing nearly to E (Fig. 12); with a strong solution of eosin all the spectrum from a

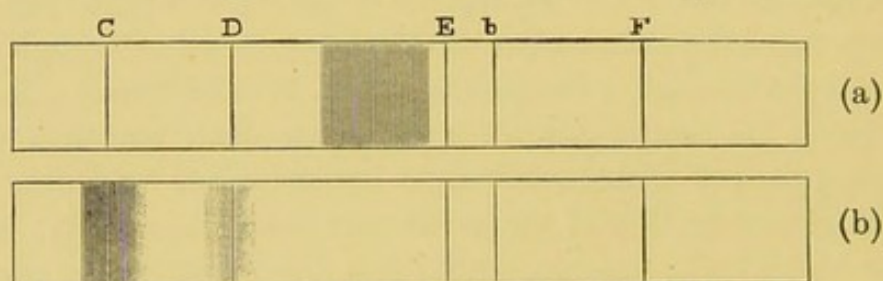


FIG. 12. Absorption spectra—(a) eosin; (b) methylene-blue.

little beyond D to the violet end is absorbed. If a drop or two of a dilute acid is added to an alcoholic extract of moderate strength, the colour disappears, and, with it, the spectrum; alkalisiation restores the colour and fluorescence, and the solution again yields its spectrum.

Blue and green urines are occasionally met with, due to the individual who passes the urine having eaten some confectionery tinted with methylene blue, or having ingested the dye in some other manner. For reasons given in the section on indoxyl in urine, the spontaneous occurrence of indigo-blue in fresh urine is very rare; therefore, urine which has a blue tint when voided should always excite suspicion of being artificially pigmented. This remark applies still more cogently to freshly-voided green urine, which, with the exceptional occurrence of oxidised bile-pigment, and the greenish

coloration after poisoning by certain substances, *e.g.*, phenol and cocaine, is probably never due to pathological conditions, but to adventitious colouring matter, usually to methylene-blue; the green colour, as stated in the section on testing the permeability of the kidneys with methylene-blue, is simply an interference phenomenon which occurs when the amount of methylene-blue is small. Weber¹ points out that when a very small amount of methylene-blue has been taken, or when most of a larger amount has been eliminated, the green appearance is usually limited to the urine which is passed on rising in the morning. When green urine due to methylene-blue is extracted with amyl alcohol, the extract is much more blue in colour than the urine was before extraction. With the spectroscope the extract from urine containing methylene-blue gives a distinct band, which spreads a little on both sides of the C line; sometimes a second faint band may be seen near to the D line.

The presence of traces of anilin colours in urine is probably not quite so uncommon, especially among young women and children, as is generally supposed, since there may be nothing striking in the appearance of the urine to attract attention. For example, a specimen of urine passed by a little girl appeared quite natural when poured into a test-tube; but on glancing down the tube through a layer two or more inches deep, a peculiar reddish-brown appearance was observed. The extract obtained from it with amyl alcohol was lilac-coloured, and it gave a faint band extending from D a little towards C, which corresponded with the spectrum of a weak solution of methyl-violet. On investigation, it was found that the child had been eating sweets coloured with this dye.

The presence of these pigments in urine does not indicate any injury to health; but when the coloration is distinct the unnatural appearance of the urine gives rise to anxiety in the minds of parents whose children are thus affected.

Some substances which may be accidentally present in urine do not interfere with its usual colour unless the urine is treated with certain reagents.

Iodine.—When an iodide or iodoform is taken by the mouth, or after the external application of iodoform to wounds, or of tincture of iodine to the unbroken skin, iodine in combination appears in the urine. On testing urine for albumin with nitric acid, a brownish-red coloration often appears immediately above the layer of acid; such an appearance may be due to the oxidation of a urinary chromogen, but a very similar appearance in the urine of patients who are taking potassium iodide, or iodine in some other form, may

¹ *The Lancet*, 1901.

be caused by the liberation of the iodine from its bases. The distinction is readily made by agitating urine thus tinted with a little chloroform: iodine is at once dissolved by the chloroform, to which it imparts a rosy-pink colour, whereas the urinary pigment is taken up slowly, if at all, and any colour imparted is not rosy pink.

For the detection of iodine, a little chlorine- or bromine-water is added to the urine, which is then shaken with either chloroform or carbon bisulphide; the free iodine is taken up by the solvent to which it gives a pink or rosy-red colour. If the amount of iodine is very small it may not reveal itself by this method. In such a case the urine, after being made strongly alkaline with potash or soda, is evaporated to dryness and the residue is carbonised; the carbonised product is extracted with water and the extract is tested as above described. Ether must not be used as a solvent, as the colour imparted to it by iodine is brownish-yellow, like that afforded by bromine.

Bromine.—After the administration of bromides, bromine in combination appears in the urine; unless it is present in considerable quantity, however, that is to say, more than is usually present, it does not respond, like iodine, to tests directly applied to the urine. A little sodium carbonate is added to some of the urine, which is then evaporated to dryness and the residue carefully carbonised; the heat should be sufficient to render the organic matter insoluble, but it should not be excessive. The carbonised product is extracted with a small quantity of water, a little chlorine-water, or strong hydrochloric acid is then added, and the liberated bromine is dissolved out in some carbon bisulphide, or chloroform which it tinges a brownish-yellow.

Jolles¹ proposes the following test: To 10 c.c. of the urine in a small flask, a little sulphuric acid is added and a sufficiency of potassium permanganate as to produce a permanent red-coloration. A slip of paper moistened with di-methyl-phenylendiamine (0.5 gm. to 500 c.c. water) is suspended in the neck of the flask, which is then warmed. If only a trace of bromine is present the paper is coloured red-violet.

Drugs of coal-tar derivation cause the urine of patients who are taking them to give well-marked colour reactions with appropriate reagents:

Salicylic acid, salol, and aspirin cause the urine to become purple or brownish-red on the addition of a few drops of a solution of ferric chloride; the colour becomes deeper when the urine is

¹ *Zeitschr. f. analyt. Chem.*, 1898.

heated. With a solution of copper sulphate an emerald-green colour is produced. The urine freely reduces Fehling's solution.

Guaiacol and **Izal** may be detected by gently warming the urine with about one-fourth its volume of hydrochloric acid, and when cold shaking it with ether; the residue left by the evaporation of a little of the ether gives a bluish-green coloration with a drop of a weak solution of ferric chloride.

Phenacetin.—The urine should be boiled with a little hydrochloric acid and, when cool, treated with some 3 per cent. solution of phenol, and then with a little bleaching-powder; a red colour is produced which, when the solution is alkalisied with ammonia, changes to blue.

Antipyrin.—On the addition of ferric chloride the urine becomes brownish-red; the colour is increased by boiling. The urine does not reduce Fehling's solution.

Alcohol.—In cases of acute alcoholic poisoning, the presence of alcohol in the urine may sometimes be detected by adding a few drops of solution of potassium dichromate to a little of the urine in a test-tube, and then running down the side of the inclined tube a cubic centimetre or two of strong sulphuric acid; a green coloration is produced at the junction of the urine and the acid. When less alcohol is present it is necessary to distil some of the urine and to test the distillate, either as above described, or by adding some strong aqueous solution of iodine, followed by sufficient solution of potash to decolorise it, and then heating the test-tube in the spirit-lamp; either at once, or on cooling, a cloud of iodoform appears, which may be recognised by its odour and by the form of the crystals—like those of cystin, or else like rosettes—as seen under the microscope. It is to be remembered that acetone yields the same reaction.

Chloral hydrate may appear in the urine of those who are taking the drug, but more constantly urochloral acid is present; urine containing it reduces Fehling's solution, and has a slight lævo-rotatory power. The acid may be isolated by evaporating the urine to one-fourth its volume, acidulating with hydrochloric acid, and shaking with ether, which on evaporation leaves needle-shaped crystals. When dissolved in a little water the crystals reduce Fehling's solution.

Chloroform.—Urine that contains chloroform reduces Fehling's solution. The presence of small amounts of chloroform is best detected by distilling some of the urine and then heating the distillate in a flask, and drawing the vapour through an incandescent glass tube, by which the chloroform-vapour is split up into chlorine and hydrochloric acid: the former may be recognised by holding a

piece of starch-paper, moistened with a weak solution of potassium iodide, to the free end of the tube, when the liberated iodine turns the starch-paper blue; hydrochloric acid may be recognised by the reddening of blue litmus-paper similarly placed, and by allowing the vapour to pass through a solution of silver nitrate, which is changed into silver chloride.

Quantitative estimation may be made by means of Waller's¹ process.

Urotropine.—If a little bromine-water is added to the urine of a patient taking urotropine, an orange-yellow precipitate is formed. The reaction can be obtained fifteen minutes after the drug is taken. (Nicolaier².)

ADVENTITIOUS METALLIC SUBSTANCES.

Lead.—Of any lead which is accidentally received into the system only a small proportion is excreted by the kidneys, the greater part is eliminated by the bowels. The lead that is present in urine is organically combined and must be dissociated from its organic moiety before it will respond to the usual tests. The urine is evaporated to about one-fourth its volume, and is placed in a large flask along with a few crystals of potassium chlorate and some hydrochloric acid. The flask is then gradually heated on the water-bath until the solution becomes of a faint yellow colour, more chlorate and hydrochloric acid being added if necessary; but neither should exceed the smallest amount that will produce the desired result. The solution is transferred to a porcelain basin, which is allowed to remain on the water-bath until the odour of chlorine has entirely disappeared; it is then filtered hot, and a clear, slightly tinged filtrate is obtained. The amount of lead in the urine is not likely to exceed that which will remain in solution as lead chloride; the substance on the filter, however, should be tested for lead in order to see whether any has been left behind. When cold, the filtrate is put into a glass cell, the bottom of which consists of a sheet of vegetable parchment; this cell is placed in an outer cell containing distilled water acidulated with a few drops of sulphuric acid, so that the liquids in the inner and the outer cells stand at the same level. A cathode of platinum foil is placed in the liquid contained in the inner cell and an anode of the same size, also of platinum, in the outer cell; through them is passed a current of about three or four volts in the direction indicated, the circuit being closed for six or eight hours. The foil from the inner

¹ *Brit. Med. Journ.*, 1901.

² *Zeitschr. f. klin. Med.*, 1899.

cell is washed and dried, and the lead, dissolved off with dilute nitric acid, is converted into sulphate, ignited, and weighed: 100 parts equal 68.319 of metallic lead. When identification only is required the solution of lead in nitric acid is evaporated to dryness, and the residue, dissolved in distilled water, is submitted to the usual tests: sulphuretted hydrogen produces a brownish colour and potassium iodide a yellow colour.

Copper.—After destruction of the organic matter, as above described, the solution is submitted to electrolysis, and the copper, dissolved off the platinum with dilute nitric acid, may be tested in the usual way: a drop of a solution of potassium ferrocyanide gives a brown colour. The amount of copper may be estimated volumetrically.

Arsenic may be most readily detected by Reinsch's method. The urine, evaporated to about one-fourth in volume, is put into a flask with one-fifth its volume of hydrochloric acid and a small piece of copper foil; the flask is then placed on a gauze-covered tripod and its contents are quietly boiled for half an hour, or longer if the amount of arsenic is very small. The copper is removed and, after being successively washed with water, alcohol, and ether, is dried on filter-paper, and is then placed in a small sublimation-tube and gently heated until the film of arsenic is volatilised. The arsenical vapour combines with oxygen and is deposited in the cooler part of the tube in the form of octahedral crystals of arsenious oxide which may be recognised under the microscope; they may also be tested with ammonio-nitrate of silver, which produces a yellow colour due to the formation of silver arsenite. Before testing the urine, the reagents should be tested for arsenic in the same flask that is subsequently used. A small glass funnel may be placed in the neck of the flask to retard evaporation when prolonged boiling is required.

Mercury may be dealt with in the manner described for arsenic. The deposit obtained after volatilisation in the sublimation-tube takes the form of minute globules of metallic mercury which are easily recognisable under the microscope. Into the cold sublimation-tube a small fragment of solid iodine may be dropped, when a faint yellow coloration soon appears in the part of the tube that is occupied by the mercury; in a little time the colour changes to the bright red of mercuric iodide.

If a quantitative estimation of mercury is desired the same method may be pursued as with lead, a piece of gold foil as the cathode being substituted for the platinum. After the mercury is deposited the gold foil is washed with water, with absolute alcohol,

and lastly with ether; it is then carefully dried and weighed. After weighing it is introduced into a piece of hard-glass tubing through which a current of dry air is passed, and heat is applied to volatilise the mercury and to cause it to deposit on the walls of the tube. The foil is re-weighed, and for control purposes the tube is weighed with the deposit, and again after the mercury has been driven off by heat.

SPECIAL CHARACTERISTICS OF URINE.

REDUCING POWER.

NORMAL urine possesses a certain degree of reducing power, and also slightly rotates the plane of polarised light to the left, characteristics which are generally admitted to be due to the presence in the urine of conjugated glycuronic-acid compounds.

The reducing power of normal urine is thus estimated by Rosin :¹ 25 c.c. of the urine, diluted with five volumes of water and with 1 c.c. of liquor potassæ added, are put into a 100 c.c. flask. The solution, covered to about three times its height with paraffin oil, is carefully boiled, and with the aid of a pipette 1 c.c. of a solution of methylene-blue (1 : 3000) is added below the paraffin; the colour is at once destroyed by the reducing action of the urine. A sufficiency of a 1/100 normal permanganate solution is then run in below the paraffin by means of a burette with a long tube as to cause the blue colour to return; the amount of permanganate solution used represents the reducing power of the urine. Gregor² uses a slight modification of Peška's method, which consists in heating to 80° or 85° C. 100 c.c. of an ammoniacal solution of copper sulphate, Rochelle salt, and sodium hydrate (much the same as Pavy's solution) covered with a layer of paraffin oil, and then gradually adding beneath the surface of the oil a 1 per cent. dilution of the urine to be tested, until the blue colour of the cupric-oxide solution disappears. Gregor found, as a result of the food that is eaten, that the reducing power of normal urine varies from 0.0825 to 0.347 per cent. in the course of the twenty-four hours; during inanition the reducing power remains constant, being equal to about 0.085 per cent. Increase in the amount of carbohydrate food has no influence, but restricted animal diet diminishes, and the use of alcohol increases, the reducing power. Metabolism and the reducing power of urine stand in inverted relations.

¹ *Münchener med. Wochenschr.*, 1899.

² *Centralbl. f. d. Krankh. d. Harn-u. Sexualorgane*, 1899.

OXIDATIVE POWER.

A recently discovered class of soluble ferments which possess the power of acting as oxidising agents—oxidases, as they have been called—have been found in various animal and vegetable tissues. Some of these ferments, unaided, turn freshly prepared tincture of guaiacum blue; others only do so with the aid of an oxygen-yielding reagent. Bourquelot¹ divides these substances into direct oxidases which take oxygen from the air and afterwards yield it to oxidisable bodies; and indirect oxidases which only act by setting free a portion of the oxygen of an aqueous solution of hydrogen peroxide, the oxygen thus liberated also combining with oxidisable bodies. Indirect oxidases are stated by Carrière² to be present in certain pathological urines—in cases of pneumonia, epilepsy, purpura hæmorrhagica, cancer, acute rheumatism, Bright's disease, tuberculosis, and other diseases—but not in normal urine.

PROTEOLYTIC POWER.

Normal urine contains traces of pepsin, and attempts have been made to utilise this fact in the diagnosis of those diseases of the stomach that are associated with irregularities in the secretion of the gastric juice. The proteolytic power is estimated by allowing a measured quantity of urine to act on a known amount of proteid substance, and then ascertaining the percentage of the proteid that has been peptonised by the enzyme in the urine. For this purpose Troller's³ method, founded on that of Hammerschlag, is very convenient and delicate. One gramme of protogen [an albuminoid substance which in aqueous solution is not coagulated by heat] is dissolved in 100 c.c. of 2 per cent. hydrochloric acid. Of this solution 10 c.c. are mixed with 3 c.c. of the urine to be tested and the mixture, in a test-tube, is placed in a cultivation-chamber at 37° C. for twenty-four hours. In another tube of the same size a control experiment is made with 10 c.c. of the acid protogen solution, 3 c.c. of water being substituted for the urine. At the end of the twenty-four hours the specimens are cooled and each is treated with 5 c.c. of Esbach's reagent; the respective amounts of precipitates are then compared; the difference indicates the peptonising power of the urine. The urinary salts exercise no inhibitory effects on the action of the ferment. According to Troller's investigations there is a distinct parallelism between the secretory activity of the peptic glands and the amount of enzyme in

¹ *Compt. rend. Cong. Internat. Med.*, 1897. ² *Compt. rend. Soc. Biol.*, 1899.

³ *Arch. f. Verdauungskrankh.*, 1899.

the urine. Friedberger¹ by the same method corroborates Trollei's results. He found that the amount of pepsin present in the urine is dependent upon the amount secreted by the gastric glands: a small amount in the urine is indicative of faulty secretion by the stomach. In health, exceptional irregularities occur in the amount of pepsin in the urine; in hyperchylia the amount thus secreted is both relatively and absolutely considerable, yet the difference is not sufficiently pronounced as to make it of diagnostic value. On the other hand, in cases of abnormally diminished secretion of gastric juice, the reduction of the amount of pepsin in the urine is so clearly manifested as to constitute an important aid in the diagnosis of such cases.

TOXICITY.

Toxic effects have long been known to follow the injection of urine into the blood-current of animals, and much discussion has ensued as to the mode in which these effects are produced. Some writers regard them as essentially toxic in the usual meaning of the term; others consider that the physical condition of the injected urine accounts for most, if not all, of the symptoms that occur.

In 1881, Feltz and Ritter² came to the conclusion that the toxic action of normal human urine is due to the potassium salts it contains. Herringham³ also believes that there is no valid ground for concluding that any urinary constituent but potash is actively concerned in the production of the toxic symptoms. Bouchard,⁴ whilst admitting that the potassium salts occupy a prominent position among the poisonous constituents of urine, attributes considerable potency to the various organic substances which it contains, and has evolved an elaborate mode of estimating the degree of toxicity possessed by urine. He assumes that the average fatal dose of normal human urine to a rabbit weighing one kilogramme is equal to 60 c.c.; this he calls a "urotoxy"; the amount of poison daily excreted is expressed in the kilo-weight of the individual, the equivalent of each kilo representing the "urotoxic co-efficient." The so-called normal "urotoxic co-efficient" has been estimated at from 0.25 to 0.49. Under pathological conditions it is stated to have reached 2.18, and even higher.

Other observers attribute the toxicity of urine solely to its organic constituents, and record the occurrence of increased toxic potency in various diseases: diphtheria, acute typhlitis and peritonitis, cholera, septicæmia, tetanus, scarlet fever during the febrile stage and for two or three days after the crisis, and in various forms of

¹ *Zeitschr. f. klin. Med.*, 1900.

² *De l'Urémie expérimentale*, 1881.

³ *Journ. Path. and Bacteriol.*, 1899.

⁴ *Compt. rend. Acad. des Sc.*, 1886.

insanity. Some experimenters state that, by means of the ordinary toxicological methods used for the separation of the vegetable alkaloids from animal matter, or by means of Brieger's process for the isolation of animal alkaloids, they have obtained substances—some in the crystalline and others in the liquid form—which respond to the alkaloidal group reagents, and which in some instances, when injected into mice and other small animals, produced various toxic symptoms. These substances have been classified as **Ptomaines**. Whilst it is to be admitted that traces of abnormal basic products are from time to time present in pathological urines, the limits of credence are exceeded by the reported isolation, from a litre or two of urine, of definitely constituted, morbid substances in the pure, crystalline form, in such amounts as to permit of quantitative elementary analyses being made from which their molecular formulæ have been deduced, their chemical reactions determined, and their poisonous effects experimentally investigated. This, it is stated, has been accomplished in a considerable number of diseases, the "ptomaine" allotted to each disease having its own formula, which differs from all others. To a group of basic substances present in normal urine, the term "leucomaine" has been applied; this is merely another name for creatinin, the xanthin bases, and some of the other urinary organic basic substances.

Recently, there is a disposition on the part of some German and French writers to explain the toxic effects produced by the intravenous injection of the considerable quantities of urine which are used in these experiments, on purely physical grounds. They maintain that absence of isotony between the injected urine and the blood serum exercises such a disturbing influence on the osmotic pressure of the blood as to account, in a great measure, if not entirely, for the symptoms produced. As evidence of this it is stated that solutions of common salt, or of grape-sugar, which have the same osmotic pressure (measured by the lowering of the freezing-point) as urine, and administered in the same doses, produce equal toxic and lethal effects. Further, that these solutions and urine itself, when made isotonic with blood, are harmless.

The inference to be drawn from these investigations is, that little has been established beyond the fact that the injection of urine into the blood current of the smaller animals produces toxic and lethal results. The methods are surrounded by too many fallacies to allow them to be used for the purpose of accurately determining the toxic potency of urine.

The apparatus for the performance of urine-injection into the blood-current is extremely simple. By means of a rubber tube a fine aspirating-needle is connected with a burette, in the upper end

of which is a stopper furnished with a small tube, and through it air is forced, so that a regular flow of urine through the needle can be produced. The needle is introduced into a conveniently situated superficial vein of a rabbit, and the injection is then slowly made.

MOLECULAR CONCENTRATION.

KRYOSCOPY.

The freezing-point of water is lowered by the presence of any substance that it holds in solution, and the extent to which it is lowered is proportional to the molecular concentration of the dissolved substance. The application of this law enables an accurate determination to be made of the concentration of the blood and of the urine. When the kidneys are deficient in functional activity the molecular concentration of the blood is increased by the effete products which, through defective elimination, are retained by it; as a natural result, the molecular concentration of the urine is concurrently diminished. The exact measurement of the freezing-points of the blood and of the urine, therefore, affords an accurate means of ascertaining the efficiency or the insufficiency of the work done by the kidneys. To this method the term *Kryoscopy* is applied.

Under normal conditions the freezing-point of the blood serum is much more constant than that of the urine; subject to very limited variations, which, according to Korányi,¹ do not exceed 0.03° C., it stands at -0.56° C. The variations in the freezing-point of normal urine are much wider. Korányi² states that the freezing-point of the urine secreted by a healthy man ranges between -1.3° and -2.2° C. Lindemann³ states that it may reach as high as -0.9° , or be depressed to -2.73° . The depression in the freezing-point is usually indicated by the sign Δ , hence the freezing-point of blood would be expressed by $\Delta 0.56$; sometimes δ is applied to blood and Δ to urine.

Proportional to the molecular concentration of a liquid is its osmotic pressure, so that when the freezing-point of blood serum is below the normal, the osmotic pressure of the blood is increased. An increase in the osmotic pressure of the blood exercises an important influence on the rate of interchange between it and the tissues; in uræmia, for example, the blood is overcharged with effete products which the kidneys are unable to remove; this condition expresses itself by lowering the freezing-point of the blood from $\Delta 0.56$ to $\Delta 0.62$ or 0.70 , the freezing-point of the urine being proportionally

¹ *Orvosi Hetilap*, 1898.

² *Zeitschr. f. klin. Med.*, 1897.

³ *Deutsches Arch. f. klin. Med.*, 1899.

elevated. Lindemann found that the same group of symptoms that are encountered in uræmia may be produced in animals by the injection of concentrated saline solutions into the blood-current.

The information afforded by the lowering of the freezing-point of the blood may be of supreme importance in relation to the performance of surgical operations: the removal of a diseased kidney would be contra-indicated were it found that Δ was materially lower than Δ 0.56. Under such conditions it would be safe to infer that the assumed healthy kidney might not be physiologically active, and consequently that it might be incompetent to undertake the work of both kidneys; moreover, a kidney that is deficient in functional activity, although not to the extent of rendering it unfit to do double duty, not unfrequently abruptly ceases to act in consequence of the shock produced by the removal of its fellow. Rumpel¹ points out that kidney-insufficiency, though prohibitive of nephrectomy, does not necessarily contra-indicate the performance of an operation for the removal of a renal calculus, or for the draining of a suppurating kidney. It is to be observed that the functional activity of the kidneys cannot be reliably ascertained by taking the freezing-point of the urine alone; the wide limits between which the molecular concentration of the urine ranges in people who are in a healthy state makes it impossible to establish a normal standard. It is further to be observed that, when one kidney is diseased and the other is healthy and physiologically active, no disturbance in the molecular concentration of the blood and of the urine necessarily occurs. Although, under ordinary conditions, kryoscopy as applied to urine may be of little value, it is capable of yielding important information when associated with catheterisation of the ureters, and it is in cases of one-sided kidney disease that kryoscopy of the urine alone may be useful. When the molecular concentration of the urine from one kidney can be compared with that of its fellow, their respective functional activities can be ascertained. Casper-Richter² has shown that, when both kidneys are healthy, the molecular concentration of the urine separately delivered by them is exactly the same; any defect in functional activity of one kidney is revealed by diminished molecular concentration of its secretion. The urine from the diseased kidney will also contain less urea than that from the healthy organ.

A number of observations have been made in relation to the freezing-point of urine in various diseases, but they are of little practical value.

¹ *Münchener med. Wochenschr.*, 1903.

² *Berlin. klin. Wochenschr.*, 1899, 1900.

The freezing-point of urine or of blood may be determined by means of Beckmann's¹ apparatus for the estimation of molecular weights by the freezing-point method. The urine or blood is placed in a test-tube which, by means of a rubber disc that surrounds its upper end, is suspended within a larger tube in such a manner as to leave an air space between the inner and outer tubes. A special thermometer with a range of only 6° C., each degree being divided into hundredths, is passed through a stopper which closes the inner tube until its bulb is completely submerged in the liquid to be tested. Alongside the thermometer is a "stirrer" of platinum wire, by means of which the urine or other liquid is continuously kept in movement during the freezing process. The outer tube is then placed in a vessel containing a freezing mixture of ice and common salt. After the liquid is frozen, a few minutes should be allowed for the mercury to settle to its permanent position. The difference between the freezing-point thus obtained and that of distilled water (which has been previously accurately ascertained by the same means and with the same thermometer) indicates Δ , the depression of the freezing-point in the liquid that has been examined.

CONDUCTIVE CAPACITY OF URINE.

The electrical conductive capacity of urine affords a criterion of the ions it contains, and has been appealed to, along with kryoscopy, in order to ascertain its molecular concentration, but for several reasons the results are uncertain; amongst others is the essential difficulty that the mixture of successive portions of urine which are excreted during the twenty-four hours, each with a different reaction and composition, determines an interchange of ions and thus alters the number of molecules. The conductivity of normal urine ranges over somewhat wide limits and is much influenced by the salts it contains; broadly stated, the resistance of urine varies with its specific gravity and with the amount of saline substances present, especially sodium chloride; much sodium chloride indicates a high conductive capacity; urea has but a limited influence. Koepp² found that the withdrawal of salts materially lowered the conductive capacity of a given specimen of urine: at 18° C. a certain urine had a conductive capacity l equal to 270.9.10⁻⁸ Ohm; after being cooled to -2° C. it was filtered and warmed again to 18° C., when l equalled 264.0. Tereg³ states that in pneumonia the resistance is high on account of the absence of chlorides, and that it is also high in diabetes notwithstanding the sugar that is present.

¹ *Zeitschr. f. physikal. Chem.*, 1888.

² *Berliner klin. Wochenschr.*, 1901.

³ *Arch. f. Physiol.*, 1901.

CALORIMETRY OF URINE.

When organic matter undergoes combustion in the presence of excess of oxygen, each ultimate constituent assumes its highest permanent state of oxidation: carbohydrates yield carbon dioxide and water; proteids yield nitrogen, carbon dioxide and water; and so on with other organic bodies. In the process of oxidation, the inherent potential energy of these bodies expresses itself as heat which, when measured, indicates in calories the combustion-value (latent energy) of the substance under examination. The term "calory" is used to express the heat developed by the combustion of one gramme of an organic substance; the amount of oxygen with which it combines during combustion indicates its oxygen-capacity. Voit¹ finds that the oxygen-capacity of almost all organic combinations bears a close relation to their combustion-heat; for all members of the same group of organic substances it is almost constant. The method of investigation founded on these lines is named "calorimetry."

In their nutrient capacity food-stuffs are endowed with potential energy (latent heat) which is set free by the changes they undergo in the processes that precede, that accompany, and that follow assimilation. A variable, unutilised percentage is excreted in the urine and the fæces; the amount of available energy thus wasted can be estimated calorimetrically, and, by comparative observations, an accurate inference can be drawn as to the efficiency of the systemic metabolism. The presence in the urine of an excessive amount of latent energy indicates an equivalent loss of nutrition due, either to abnormal derangement of metabolism, or to faulty character of the food.

By means of the calorimetric method, Tangl² determined, in the human subject, the relation borne by the energy-capacity of urine to its nitrogen and carbon value; and also how this relation is affected by special diet and by exercise. For several consecutive days the subjects of the experiments lived chiefly on fatty food, and then chiefly on carbohydrates for a like period; they took exercise and rested alternately, the urine after exercise and after rest being separately collected and examined. The results showed that the kind of food exercises a considerable influence on the calory quotient $\frac{\text{Cal}}{\text{N}}$, and on the carbon quotient $\frac{\text{C}}{\text{N}}$ of urine, which were greater when the food was chiefly carbohydrate than when chiefly fatty; with chiefly carbohydrate food the $\frac{\text{Cal}}{\text{N}} = 11.93$, the $\frac{\text{C}}{\text{N}} = 0.944$; with chiefly fatty food, 8.59 and 0.691 respectively. On the other

¹ *Zeitschr. f. Biolog.*, 1903.

² *Arch. f. Physiol.*, 1899.

hand, the quotients were not disturbed by the alternations of exercise and rest; a result which agrees with Zuntz's theory that "the same admixture of food-stuffs is dealt with during rest and during work." Schlossman¹ urges the use of the calorimetric method in clinical investigations, especially in diseases in which the metabolism is essentially deranged, such as diabetes and gout, and also in renal diseases. Much valuable information may doubtless be obtained by calorimetry applied to the urine; but, although the method is not exceptionally difficult, it lies a little wide of the customary methods of clinical investigation, and requires apparatus which is not found in the ordinary clinical laboratory.

The determination of the combustion-heat is accomplished either by means of a Berthelot-Mahler calorimetric bomb, or with the aid of the apparatus devised by Hempel.² For description of the methods, the papers quoted must be consulted.

EHRlich'S DIAZO-REACTION.

To 10 c.c. of urine an equal volume of a saturated solution of sulphanilic acid in 5 per cent. hydrochloric acid is added, along with a couple of drops of a half per cent. solution of sodium nitrite; the solution is then made alkaline with ammonia. A positive reaction is indicated by the liquid becoming bright crimson in colour, and the froth, on shaking, pink or salmon-coloured.

The substance in urine to which this reaction is due is not known, and the value of the reaction itself has been variously estimated; the tendency is to regard it as being of less value as an aid to diagnosis than to prognosis.

Diagnostic Indications.—A positive reaction is usually given by the urine from cases of enterica, and has been regarded as indicative of that disease; it usually disappears during defervescence and returns should a relapse occur. A lower reaction-rate than usual was observed in enterica by Gebauer³: out of fifty-eight cases of enterica, a positive reaction was obtained in thirty-nine, that is, in 68.99 per cent.; a persistent negative reaction occurred in seventeen, or 29.31 per cent., and in two instances the reaction was doubtful; so that in 31.03 per cent. of the cases the results were useless for diagnostic purposes. It is obtainable in about 80 per cent. of the cases of measles. It occurs in various forms of tuberculous disease, and may be utilised in diagnosing between intestinal

¹ *Berlin. klin. Wochenschr.*, 1903; *Zeitschr. f. physiol. Chem.*, 1903.

² *Zeitschr. f. angewandte Chemie*, 1901.

³ *Vierteljahrsschr. f. öffentliches Sanitätswesen*, 1903.

tuberculosis and malignant disease; a negative reaction points to malignant disease. In the miliary tuberculosis of children, whether abdominal or cerebral, the reaction is almost constant. It appears in puerperal septicaemia, and in the course of septic complications in other diseases, such as diphtheria and scarlet fever. It is met with in many cases of pneumonia.

Blumenthal¹ points out that the reaction may be utilised to distinguish between the rashes produced by drugs and those of exanthematous diseases. The eruptions caused by salicylic acid, iodine, antipyrin, and belladonna, as well as those which sometimes occur after crabs, mussels, and other shell-fish are eaten, along with the enema rash, are never accompanied by the diazo-reaction; if the reaction occurs it points to an eruptive disease, all forms of which, however, do not yield the reaction.

Prognostic Indications.—In acute tuberculosis the occurrence of the diazo-reaction is a bad sign; of thirty-six tuberculous patients who did not yield the reaction three died; of one hundred and eight who yielded the reaction eighty died (Michaelis²). The intensity of the reaction is said to be not without prognostic value: in enterica, and also in influenza, it has been found to be proportional to the severity of the disease.

Certain drugs, such as morphine, chrysorobin, and naphthalin, produce the reaction; whereas others, such as gallic acid, phenol and its derivatives, cresol and guaiacol, tend to inhibit it (Burghart³).

METHYLENE-BLUE TEST.

When injected subcutaneously, methylene-blue appears in a short time in the urine, a result that has been utilised for the purpose of testing the permeability or the functional activity of the kidneys. In the healthy state, the hypodermic injection of 0.05 grm. of methylene-blue is usually followed by a blue coloration of the urine in about thirty minutes; when the amount of pigment in the urine is very small the colour of the urine is green; this is merely an interference phenomenon due to the natural colour of the urine. Before the coloured urine appears, that is, in fifteen to twenty minutes after administration, the urine contains a colourless reduction-product derived from the methylene-blue, which is probably produced in the liver. Such colourless urine, if boiled with acetic acid, becomes blue or greenish; the urine which is blue when voided often deepens in colour with the same treatment, indicating that in

¹ *Pathol. des Harnes*, 1903.

² *Deutsche med. Wochenschr.*, 1899.

Berlin. klin. Wochenschr., 1899.

addition to the pigment some of its chromogen is also present. After the administration of methylene-blue the urine regains its natural colour in from twenty-four to forty-eight hours; sometimes it takes longer; the chromogen continues to be present in the urine for some time after the pigment has ceased to appear. Delayed excretion of the pigment—manifested either by prolongation of the interval between its injection and its appearance in the urine, or by extension of the usual period of complete elimination, or of both—is supposed to indicate renal insufficiency.

A large number of investigations with the methylene-blue test have been made, but the results are not satisfactory; some observations, however, are very suggestive. Devoto¹ found, in two individuals in whom the excretory function of the kidneys was very dilatory, that when methylene-blue and caffein were injected simultaneously the urine became blue in fifteen and in twenty-five minutes respectively. In a case of nephritis with uræmia, Widal² found that methylene-blue was excreted as usual; with this my own experience coincides and leads me to regard the test as untrustworthy.

Mattirolo³ obtained some interesting results in a number of different diseases of the liver. He found that the duration of excretion of methylene-blue is shorter than in health, and that the chromogen is present in the urine long after the pigment has ceased to appear. The excretion is not continuous: intervals of from two to six hours often occur in which no pigment but only the chromogen is excreted. Mattirolo states that the excretion of methylene-blue and that of the urea and other soluble substances of the urine does not occur on parallel lines.

PHLORIDZIN TEST.

Another and probably more reliable test for estimating the functional activity of the kidneys than the methylene-blue test is proposed by Achard and Delamare.⁴ They utilise Klemperer's observation that the injection of phloridzin produces no glycosuria in patients who are suffering from kidney disease. In a healthy person the hypodermic injection of 5 mgrms. of phloridzin is followed within three hours by the excretion of from 0.5 to 2.5 or, exceptionally, 6 grms. of sugar in the urine. If the function of the kidneys is deranged the glycosuria either does not occur or, if it occurs, the amount of sugar is below the minimum above given.

¹ *Gazz. degli Ospidali*, 1898.

² *Bull. de la Soc. Méd. d. Hôpit.*, 1900.

³ *Giorn. d. R. Accad. d. Med. d. Torino*, 1902.

⁴ *Compt. rend. Soc. Biolog.*, 1899.

URINARY SEDIMENTS.

THE collection of sediments for microscopical examination is facilitated by the use of cylindrical urine-glasses, the bottoms of which, interiorly, should be paraboloid, not conical in form. When a specimen of urine has stood for a few hours in a urine-glass, a more or less obvious deposit usually collects at the bottom, which may consist of organised or unorganised substances, or of both. The deposit varies in consistence from one which is so scanty and transparent as to be scarcely visible to that which is copious and dense. Its colour also varies: it may be white, or variously tinted, from pale-yellow or pink to fiery-red or dark-brown. To the unaided eye the deposit is sometimes obviously crystalline in appearance; at others it is of homogeneous, creamy consistence, such as the deposit of pus in acid urine; a sediment not very dissimilar in appearance may be due to amorphous earthy phosphates. Apart from the sediment itself, indications of its nature are sometimes visible in the upper stratum of the urine: an opalescent film on the sides of the glass with which the urine has been in contact is indicative of urates; glistening crystals adhering to the walls of the urine glass suggest uric acid; a white and more closely deposited series of crystals arranged in lines as though determined by the movements of the cloth last used to wipe out the vessel, suggest calcium oxalate, an indication which would be corroborated by the occurrence of a white layer on the surface of the nubecula, also due to calcium oxalate crystals, and known as the "powdered wig" deposit. An opalescent film on the surface of the urine suggests triple phosphates; but it may be due to cholesterin or other rarer constituents. A persistent turbidity which declines to subside may be due to bacteria either in fresh urine or in that which is undergoing decomposition; in the latter case the reaction will probably be alkaline; occasionally a deposit of amorphous urates is held in suspension by the presence of a large amount of globuline. A rarer cause of persistent turbidity would be the presence of chyle in the urine.

Unless very scanty, the ordinary urinary sediments may be left to deposit spontaneously; if for any reason early microscopical exami-

nation is necessary the urine should be centrifuged. Many urinary deposits, however, are seen to greater advantage when left to gravitate by their own weight than when forcibly driven down in the centrifuge, by which some, organised sediments especially, are too closely compacted. If the seasonal temperature be high, or the condition of the urine disposes it to rapid putrefactive changes, or it is necessary to keep the urine for future examination, a preservative such as formal or thymol may be added; chloroform is sometimes used but is less efficacious. May¹ states that when formal is added to urine a deposit of di-formaldehyde-urea is not unfrequently produced, which takes the form of small spheroids, not unlike crystals of calcium carbonate or of leucin. Chronheim² considers that the best preservative for urine is a 10 per cent. alcoholic solution of thymol, or a saturated aqueous solution of sodium fluoride.

When searching for casts in urine which, on standing, is likely to deposit urates, Harris³ recommends that it should be diluted with an equal volume of a solution consisting of 60 grms. of potassium acetate dissolved in 1000 c.c. of distilled water, which is then saturated with chloroform. This method, by preventing the deposition of urates, greatly facilitates the search for casts. By means of a pipette a small portion of the sediment is transferred from the urine-glass to a microscope slide.

UNORGANISED SEDIMENTS.

URIC ACID.

When spontaneously deposited from urine the crystals of uric acid are more varied in form, size, and colour than those of any other crystalline substance that is precipitated from urine. The commonest form is the so-called whetstone and barrel-shaped crystals, represented in Fig. 13. Crystals having this form are frequently agglomerated in tufts or masses. The dumb-bell, the spear-shaped, the bar-shaped, and the fusiform are less common. Thin plates, almost colourless, are sometimes present in light-coloured urine; the plates are usually diamond-shaped, occasionally with two of the points cut off, making six-sided figures. The less common and larger forms of crystals are usually deposited from strongly acid urine; from less acid urine the whetstone form is the most

¹ *Deutsches Arch. f. klin. Med.*, 1900. ² *Arch. f. Physiol.*, 1902.

³ *Brit. Med. Journ.*, 1894.

frequent. When uric acid crystals are present in urine that has undergone alkaline fermentation they are usually eroded and misshapen.

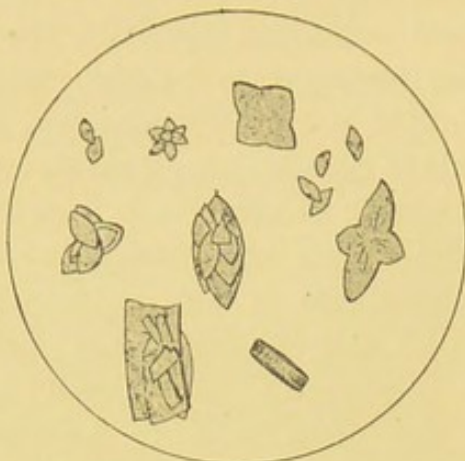


Fig. 13. Uric acid crystals
(common forms).

In whatever form uric acid crystallises out of urine the crystals are tinted by the urinary pigments, chiefly by uroerythrin for which uric acid has a strong affinity. The tint varies from pale yellow or yellowish-brown to brilliant-red, which causes the crystals to resemble, to the naked eye, particles of cayenne pepper. Exceptionally, when the urine is excessively pale, as in diabetes, any crystals of uric

acid that are deposited are free, or almost free, from colour; in cases of leucocythæmia colourless crystals of uric acid have been observed. The salts of uric acid have also an affinity for uroerythrin, but in a less degree as compared with the free acid; a pale urine from which colourless urates are deposited may also furnish crystals of the free acid that are deeply pigmented. Uric acid appears to attract and to combine with various pigmentary bodies that may be present in urine; crystals deposited from the urine of patients who are taking sodium salicylate, or salol, sometimes have a smoky tint; they have been seen to have a bluish hue produced by spontaneously oxidised urinary indican, a condition, however, that is extremely rare. In icteric urine uric acid crystals are often bile-stained.

URATES.

Amorphous urates appear as a dense sediment of a pink or bright-red colour; sometimes they are yellowish, and in children are usually white.

Sodium biurate is not a common urinary deposit. It crystallises out of urine, which is usually acid, in the form of spheres with projecting spicules, to which the name thorn-apple-crystals is applied (Fig. 14). In the crystalline form, sodium biurate is much less soluble in water than it is in the ordinary condition in which it occurs in urine. It tends to be precipitated in the colloid state and afterwards to become crystalline. The form of the crystals causes them to be very irritating to the bladder, especially in the case of children

who are occasionally thus troubled; in such cases the deposit, as it appears to the unaided eye, is often mistaken for earthy phosphates.

Ammonium biurate also crystallises in the form of spheres with projecting spines, which are likewise known as thorn-apple-crystals, and are usually met with in alkaline urine along with triple and amorphous phosphates. Sometimes they form agglomerated masses which may assume irregular, mandrake-like outlines. With transmitted light, the crystals appear under the microscope to be opaque and yellowish-brown in colour. Exceptionally, ammonium biurate crystallises in fine needles.

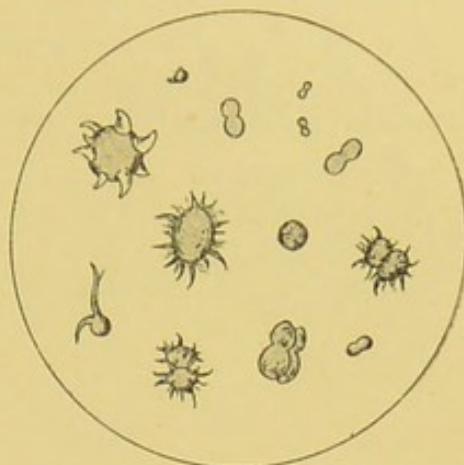


Fig. 14. Ammonium biurate crystals.

Calcium urate is a rare urinary deposit. Delépine¹ describes it as taking the form of long needle-shaped crystals, generally grouped together as spiny spheres, or it appears as an amorphous precipitate.

Chemical Reactions.—Uric acid and all urates give the murexid reaction. If acetic acid is added to sodium and ammonium urates, the uric acid is separated and crystallises out. Calcium urate is detected by the addition of a little sulphuric acid, which separates the acid from its base and produces a precipitate of uric acid and calcium sulphate. Amorphous urates readily dissolve when the urine is warmed.

PHOSPHATES.

Earthy phosphates of calcium and magnesium appear as a colourless, amorphous deposit from urines which have an alkaline, an amphoteric or a neutral reaction.

Triple phosphates (ammonium magnesium phosphate) are commonly present in ammoniacal urine, as in cases of cystitis, together with earthy phosphates and occasionally with crystals of ammonium biurate. The triple phosphate crystals are the largest found in urinary deposits; the common form is that of transparent, colourless prisms with bevelled ends. Much less frequently they appear as thin, frond-like crystals often arranged in stars and crosses (Fig 15).

¹ *Proc. Physiol. Soc.*, 1887.

Stellar phosphates, the mono-hydric calcium phosphate, is frequently found in neutral or feebly acid urines. The individual

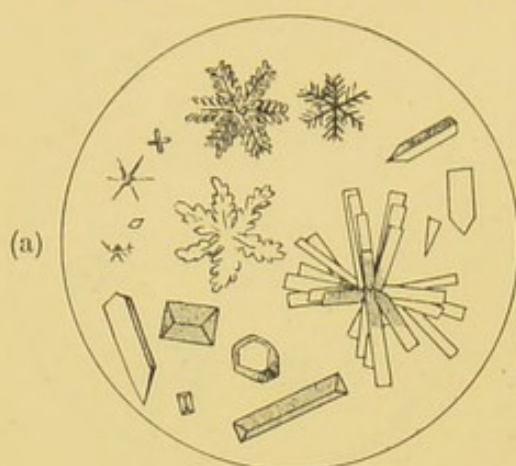


Fig. 15. (a) Triple phosphate crystals.
(b) Stellar phosphate crystals.

crystals are prismatic or rodlike, tapering to one end or bevelled like a mortise chisel; they are often concentrically arranged, forming stars (whence the name stellar phosphates), fanlike groups, and other forms. This calcium salt is sometimes found as a pellicle on the surface of urines of neutral, or nearly neutral reaction, which have stood for some

time. Very rarely it occurs in fine needles clustered in sheaves.

Mono-hydric magnesium phosphate is described by Bradshaw¹ as occurring in the urine from a patient who was suffering from dilatation of the stomach, and who took large quantities of magnesium carbonate to relieve symptoms. The urine was alkaline and effervesced with acids; it deposited long fine needles which gave the reaction of the mono-hydric salt.

Normal magnesium phosphate (Fig. 16) crystallises in rectangular plates with bevel edges, some with obliquely shaped ends; occasionally, the crystals take the form of delicate prisms or needles. Crystals of normal magnesium phosphate were first recognised in urine in 1848 by Venables,² who determined their chemical composition; he found

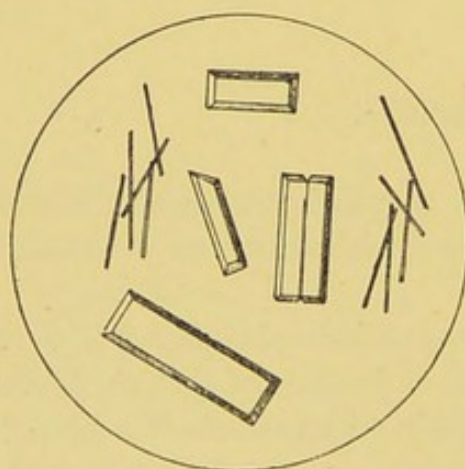


Fig. 16. Normal magnesium phosphate crystals.

on inquiry that the patient was in the habit of constantly taking a mixture chiefly composed of magnesia. The deposit has also been observed in alkaline, but not ammoniacal, urines from cases of organic and simple dilatation of the stomach, which are accompanied by profuse vomiting, and in which no magnesia has been administered; calcium phosphate and the earthy phosphates are met with under the same conditions. The prolonged

drinking of alkaline mineral waters tends to produce a deposit of magnesium phosphate. In all these cases the urine is alkaline, but

¹ *The Lancet*, 1902.

² *Med. Times*, vol. xviii.

owing to the absence of ammonia no triple phosphate is formed. In cases of dilatation of the stomach, with persistent, copious vomiting, it is easy to produce crystals of normal magnesium phosphate in the urine by the administration of half-teaspoonful doses of magnesia twice a day; an abundant deposit quickly appears.

Chemical Reactions.—All the phosphatic deposits are dissolved by acetic acid. If ammonium oxalate be added to the solution thus obtained, the calcium that may be present is precipitated as calcium oxalate; on the addition of ammonia, the magnesium phosphate is precipitated as triple phosphate.

CALCIUM SALTS.

Oxalate of lime usually crystallises out of urine in the form of octahedra, the principal axis of which is short, so that when viewed from above the octahedral angles are seen to cross diagonally a quadrilateral outline, producing the appearance known as the "envelope" form (Fig. 17). Sometimes the crystals take the form of four-sided prisms with short pyramidal ends which might be mistaken for small crystals of triple phosphate; at other times they appear like two long pyramids joined at their bases. Calcium

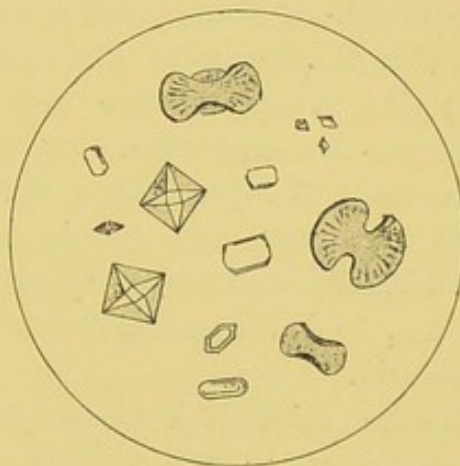


Fig. 17. Calcium oxalate crystals.

oxalate crystals also appear with curved instead of angular outlines, as twin spheroids like dumb-bells, or hour-glasses; or as thin plates with rounded ends, interspersed with which may be some with angular ends.

Chemical Reactions.—Calcium oxalate is insoluble in acetic acid; this reaction distinguishes the prismatic form of calcium oxalate from small triple phosphate crystals which readily dissolve in acetic acid. The spherical crystals of calcium oxalate may be distinguished from those of calcium carbonate by the addition of acetic acid which dissolves the carbonate with the evolution of carbon dioxide, the oxalate remaining unchanged.

Calcium carbonate is not a common deposit from human urine. When it occurs the urine is usually alkaline and coincidentally deposits phosphates. The crystals of calcium carbonate take the form of small spheres which are frequently coupled together in the hour-glass form; by the union of two of these twin crystals at a right angle a

rosette-like crystal is formed (Fig. 18). The spheres are smaller than the other spherical crystals found in urine, such as ammonium

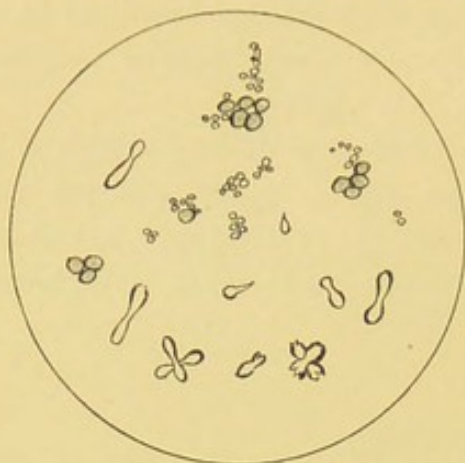


Fig. 18. Calcium carbonate crystals.

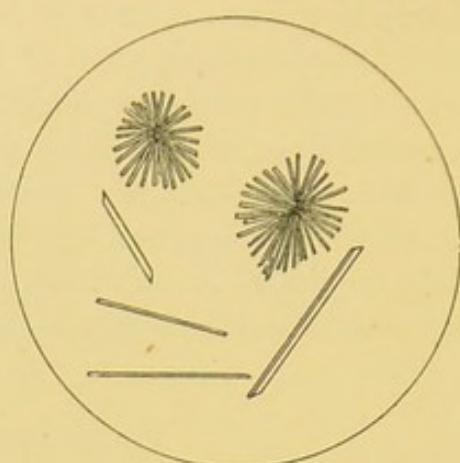


Fig. 19. Calcium sulphate crystals.

biurate, sodium biurate, and the dumb-bell calcium oxalate, from all of which they may be distinguished by their behaviour to acetic acid; they rapidly dissolve with the evolution of bubbles of gas, which are not seen when the other of the above-named crystals are so treated. The precipitate should be well washed with distilled water to remove any soluble carbonates before the acetic acid is applied. The urates are further distinguished by giving the murexid reaction, and the oxalate remains unchanged in the presence of acetic acid.

Calcium sulphate is an exceptionally rare urinary sediment. It has been found in acid urine in the form of long needles, or narrow

tabloids with bevel ends, which are distinct or are grouped in stars or crosses (Fig. 19). They are to be distinguished from the monohydric calcium phosphate crystals by their resistance to the action of acetic acid.

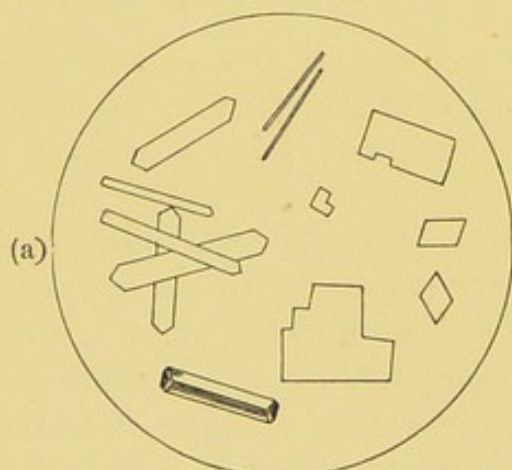


Fig. 20. (a) Hippuric acid.
(b) Cholesterin.

HIPPURIC ACID.

Hippuric acid is held by the urine in solution in combination with bases, and is rarely spontaneously precipitated in the free state. It crystallises in fine, colourless needles, or in long, four-sided

prisms or plates, which tend to group together in irregular forms; the plates are often pointed at the ends. There is a certain resemblance

between the crystals of hippuric acid and those of normal magnesium phosphate and monohydric calcium phosphate, from which they may be distinguished by acetic acid, which dissolves the phosphates and leaves the hippuric acid untouched. From bar-shaped uric acid crystals they are distinguished by the absence of colour and of reaction to the murexid test.

XANTHIN.

Although present in normal urine, xanthin is very rarely spontaneously precipitated in the crystalline form. Bence-Jones and Marcet¹ found a deposit of xanthin in the urine of a boy nine years of age, who had suffered from renal colic for three years. A few other cases are recorded. The crystals are like small, whetstone, uric acid crystals, but they appear thinner and are colourless and more uniform in size. They are distinguished from uric acid crystals by their ready solubility in dilute ammonia water.

CYSTIN.

Cystin crystallises in thin, transparent, colourless, hexagonal plates, which have a tendency to become superimposed on each other (Fig. 21). From hexagonal crystals of uric acid it may be distinguished by adding a drop of hydrochloric acid, which at once dissolves the cystin, whilst it leaves the uric acid unaltered; moreover, uric acid responds to the murexid test; cystin does not. The thin scales of calcium phosphate are not likely to be mistaken for cystin crystals; in case of doubt a drop of acetic acid will decide the question: the phosphate is at once dissolved, whilst the cystin remains unaltered.

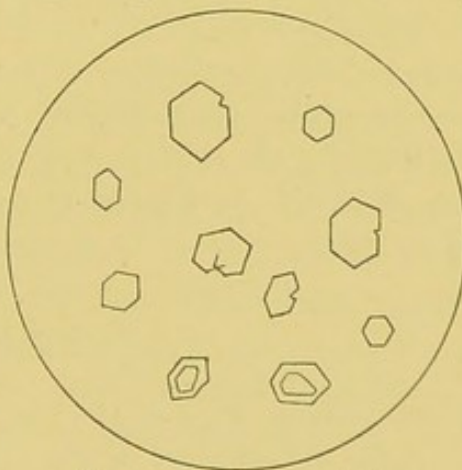


Fig. 21. Cystin crystals.

CHOLESTERIN.

Cholesterin crystallises out of urine in thin, transparent, rhombic tables, which present peculiar rectangular gaps at one or more of the corners that impart a very characteristic appearance; the plates tend

¹ *Journ. of Chem. Soc.*, 1862.

to adhere the one over the other, and thus to present irregular rectangular outlines (Fig. 20, b). If the crystals are treated with dilute sulphuric acid and a little tincture of iodine they become variously tinted—violet, blue, green, and yellow.

LEUCIN.

Leucin crystallises in small, somewhat shiny, yellow-coloured spheres, which are composed of a collection of fine needle-like crystals

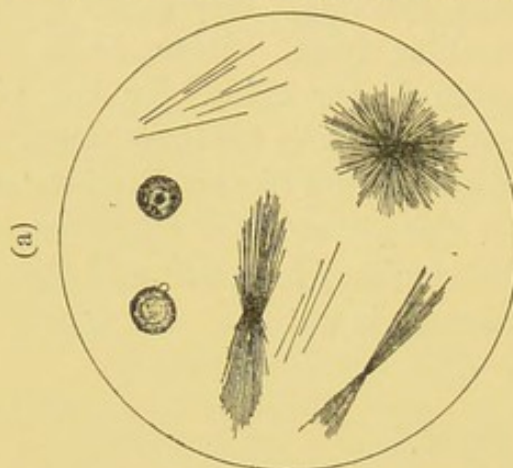


Fig. 22. (a) Leucin. (b) Tyrosin.

arranged radially round a common centre (Fig. 22, a). When seen under the microscope the typical form appears to have a ring-like periphery with radial markings; the appearance of these markings, and of others which are concentric, varies as the focus of the instrument is altered, indicating the spherical shape of the crystals.

They are distinguished from the crystals of ammonium biurate by their lighter and translucent appearance, by the striations above described, and by the absence of spicules. On the addition of a drop of dilute acid, the urate crystals disappear and give place to crystals of uric acid; they also give the murexid reaction. From fat globules leucin is distinguished by its appearance and by its insolubility in ether.

TYROSIN.

When tyrosin crystallises out of urine, either spontaneously or after simple evaporation of the urine, the crystals appear as sheaves, or tufts of fine glistening needles, which have a greenish-yellow colour (Fig. 22, b); if the urinary pigments are removed before the crystals form they are colourless. The only crystals obtained from urine, with which they might be confused, are the exceptional form of mono-hydric calcium phosphate, or possibly the equivalent magnesium salt, and calcium urate. Treatment with dilute acetic acid dissolves the phosphate salts, whilst it has little or no action on the tyrosin; a positive reaction with the murexid test reveals the presence of a urate; moreover, calcium urate is not affected by dilute hydrochloric acid, whereas tyrosin is quickly dissolved by it.

As leucin and tyrosin usually occur together in urine they may be conjointly sought for. Tyrosin being feebly soluble in aqueous liquids often crystallises out of urine spontaneously, or after moderate concentration. Leucin being very much more soluble does not separate until the urine has been evaporated to a syrup.

BILIRUBIN.

Bilirubin, or hæmatoidin, may appear in urine in the crystalline form, or in a slightly altered and amorphous condition. The crystals consist of short, reddish-brown, or yellowish needles, which are mostly arranged in crosses and stars; or they may take the form of small diamond tables, which, however, are somewhat rarer. Bilirubin crystals are easily recognised by their colour and shape. They are soluble in chloroform and benzene, and in acids and alkalies. With nitric acid they give Gmelin's reaction (biliverdin).

Crystals of bilirubin have been found in urine from cases of villous cancer of the bladder, in various hæmorrhagic conditions affecting the kidneys, in abscess of the kidney, in acute yellow atrophy of the liver, in cancer of the liver, and in several general diseases as typhoid, scarlet fever, and phthisis.

PARTICLES OF BLOOD-PIGMENT.

Not infrequently reddish-brown, amorphous particles of irregular size and outline are seen under the microscope when urinary deposits are being examined; some are entirely opaque; others appear more translucent, especially towards the margins, where they are evidently thinner and consequently the colour diminishes to brownish-yellow. In structure, these particles seem to consist of coarse or of fine granular masses which are composed of hæmoglobin-derivatives; from their insolubility in water they are probably nearer hæmatin than any of the other blood-pigments. The same substance may also be seen adhering to tubule-casts in some stages of nephritis. The free particles of pigment are met with in hæmoglobinuria; in the later stage of catarrhal nephritis in which early on there has been the usual copious hæmaturia; in renal calculus without colic, in which minute abrasions of the walls of the pelvis of the kidney occur from time to time, causing slight oozing of blood that clings to the part and undergoes chemical change before it is detached and carried away by the urine; in women shortly after the cessation of a menstrual period; and, not unfrequently, in healthy individuals without any obvious cause. Occasionally the pigment-masses are associated with hæmatoidin crystals.

INDIGO.

For reasons explained in the section on urinary indican, it is very seldom that preformed indigo is present in the urine when voided ; even after exposure to air, by no means every urine that is rich in indoxyl-salts will spontaneously yield indigo. Occasionally, however, such urines do undergo changes, especially during ammoniacal fermentation, by which the necessary oxidation is effected and the blue deposit produced. v. Jaksch¹ found indigo crystals in the urine from a case of abscess of the liver in which the urine had an acid reaction. When formed after the urine is passed, it is usually after many days' standing ; in one case Delépine² observed it in twenty-four hours. On the surface of the urine a pellicle may form which has a blue colour, often with a copper-like lustre ; the film, as in Delépine's case, may consist of phosphate-scales which contained small, short prismatic crystals, blue in colour.

Indigo blue crystallises in small deep blue prismatic crystals with a coppery lustre ; but in urinary deposits the form of the blue crystals is very varied as it depends chiefly upon the kind of deposit with which the indigo is precipitated. It may thus be associated with one or other of the phosphatic deposits, when blue crystals, either stellate, prismatic, or feathery are found ; uric acid crystals may be thus stained, and possibly those of calcium oxalate.

Indigo-blue is soluble in chloroform, and by it may be extracted from urinary sediments which contain indigo. In addition to observing the spectrum of indigo-blue in solution, which consists of a broad band between C and D, near the latter, on evaporation of the chloroform, the solid pigment may be carefully volatilised by gentle heat ; a violet-blue vapour forms (like that of iodine when similarly treated), which deposits in the form of fine, blue needles with coppery lustre.

ORGANISED SEDIMENTS.

EPITHELIUM.

The epithelium from various parts of the genito-urinary tract varies in kind, and is in some degree characteristic of the site whence it is derived ; but as the various types of epithelial cells are repeated in different situations—kidneys, bladder, and genital passages—the

¹ *Clinical Diagnosis*, 1897.

² Rieder's *Atlas*, by Delépine and Moore, 1899.

discovery of specimens of any special type in the urine is of less diagnostic value than is often supposed. When a large quantity of one kind of epithelium is present, it may be accepted as an indication that catarrhal or inflammatory processes are taking place in the part or parts whence it is derived.

In the kidney, the tubule-epithelium—mostly cubical and columnar—is small, about twice the size of a blood-corpuscle, and it has a distinct nucleus; the epithelium of the pelvis, together with that of the ureter, is globular, pyriform, or ovoid, with large nuclei; a few cuboid and columnar cells may come from the ureters. The epithelial cells from the bladder are flat, cuboid, and columnar from the superficial, middle, and deep layers respectively. Flat cells alone have no pathological significance, unless present in large numbers, as they are continuously being shed in the normal condition. The presence of cuboid and columnar cells is indicative of abnormal processes in the bladder; and when, in number, they exceed those from the surface of the lining membrane, a process of a more or less chronic nature is to be inferred. In suspected papillomata, or villous growths of the bladder, especially when undergoing ulceration, a distinct predominance of pear-shaped, or ovoid cells with one or more prolongations, which may often be observed in the deposit from the urine, is very suggestive. The flat cells of the bladder are much larger than those of any other part of the urinary tract, but they are exceeded in size by the flat cells of the vagina, which are the largest cells that are to be found in urine. The superficial squamous cells of the vulva and, in the male, those of the glans and prepuce, closely resemble the vaginal cells both in size and appearance. Any of these cells when viewed sidewise appear almost linear, with bulbous centres which represent the nuclei; when seen in the front they are often found to be aggregated in patches.

Epithelial cells are coarsely or finely granular, and have one, or more than one, nucleus. The smaller tubule-cells are not so easily distinguished from leucocytes when both are distended by imbibition, as they often are in urine; and for the same reason the distinctive contour of all the epithelial cells present in urine is liable to undergo marked alteration: thus the cuboidal cells lose their polyhedral outline and become almost or quite spherical. The intimate structure of the cells is often greatly altered by degenerative changes which take place before or after they are shed; cloudy swelling, displacement of the granular protoplasm, vacuolation, fatty changes, and the presence of pigmentary matter all tend to produce variations that may render recognition difficult or impossible.

BLOOD-CORPUSCLES.

When a very small amount of blood is present in urine, probably the only means available for its recognition will be to search for some of the red blood-corpuscles with the aid of the microscope. The appearance presented by blood corpuscles in urine varies with the density of the urine and the length of time the corpuscles have been present in it; if the urine is of high specific gravity and is examined soon after it is passed, the corpuscles may retain their natural colour and form, or they may have a crenated outline; under converse conditions they lose their normal colour and become faint shadows swollen so as no longer to appear biconcave, but rather convex or spherical in outline. These changes occur more rapidly in alkaline than in acid urines. When derived from the kidneys the blood corpuscles are usually seen to be isolated and not collected in rouleaux or clots; exceptions occur in cases of renal hæmorrhage due to calculus and to neoplasms. In vesical and urethral hæmorrhage, large or small macroscopic clots may be present in the urine, varying in size from masses that are voided with difficulty down to minute threads that are barely visible; clots are most likely to be present in cases of vesical calculus, and more particularly in consequence of villous growths of the bladder. Under any of these conditions the blood-clots may be replaced by colourless or faintly tinted fibrinous clots.

PUS.

Pus corpuscles are leucocytes that are undergoing degeneration which is often manifested by visible fatty changes. The serum of pus is an alkaline, yellowish, or greenish liquid, which contains about 7 per cent. of proteids, including a varying amount of nucleo-albumin derived from the disintegrating leucocytes. The corpuscles are colourless, spherical bodies, somewhat larger than red blood corpuscles, of a granular appearance, containing one or more nuclei which can be rendered more clearly visible by treatment with acetic acid. In alkaline urine, especially if ammoniacal, the pus corpuscles swell, lose their granular appearance, and become transparent, and finally disappear, the nucleus remaining visible to the last. In the fatty stage, which is an indication of chronic processes, the cells display a number of glistening, highly refractive points, best seen by slowly altering the focus of the microscope to and fro. Occasionally, particles of hæmatoidin, in the crystalline and the amorphous conditions, may be seen in the cells, indicating past renal hæmorrhage; at other times they are filled with black granular matter, or are diffusely

bright yellow in colour (especially when viewed in bulk), due to small amounts of blood pigment, which may be derived from the lower urinary passages.

Tests.—Leucocytes may be distinguished from epithelial cells by treatment with a little aqueous solution of iodine with potassium iodide; the leucocyte is stained mahogany-brown, whilst the epithelial cell is only tinted yellow.

If urine that contains pus (acidulated with acetic acid if alkaline) is allowed to flow through a close-textured filter, and the deposit that is left on the paper is then treated with some freshly prepared tincture of guaiacum that has not been exposed to the light, a blue coloration is produced. The oxidation of guaiacum by pus, without the help of hydrogen peroxide, is probably due to the presence of an oxidase in the pus cells (Vitali¹). When using this test, a drop of the guaiacum tincture should be allowed to fall on a fragment of the same filter-paper before the urine comes in contact with it, and the effect is observed; some filter-papers spontaneously develop a blue colour with guaiacum.

When pus, or blood, is treated with hydrogen peroxide, effervescence takes place and a stream of fine bubbles ascends through the mixed liquids. Marshall² has demonstrated that the gas which is liberated is oxygen, and believes that, in blood, the decomposing agent is globulin. Senter³ finds that it is an enzyme (hæmase), which is associated with hæmoglobin. Ville and Moitessier⁴ also regard it as an enzyme contained in the red corpuscles. This reaction of hydrogen peroxide, or of ozonic ether (by which the same results are attained) has been proposed as a distinctive test for pus; inasmuch, however, as mucus derived from catarrhal membranes yields the same reaction, the test fails at the critical point when it might be of service—to distinguish between true pus, and mucus with a few leucocytes in suspension.

Pyuria.—The significance of pus in the urine depends on the amount that is present and, more particularly, the source whence it is derived. When but a few cells are present they may represent wandering leucocytes that accompany an excessive secretion of mucus; in such a case it would probably be impossible to say whether the deposit was purulent or not; when cells are present in larger numbers, the difficulty is not so great. The appearance presented by pus characteristically differs in accordance with the reaction of the urine. In acid urine the pus forms a yellowish, or greenish-white,

¹ *Giorn. di Farm. di Torino*, 1887; *Accad. d. sc. di Bologna*, 1901.

² *Univ. Pennsylvania Med. Bulletin*, 1902.

³ *Zeitschr. f. physiol. Chem.*, 1903.

⁴ *Bull. Soc. Chim.*, 1903.

mobile deposit, not unlike that due to amorphous phosphates, the supernatant urine being fairly clear, and on agitating the vessel the pus tends to distribute itself throughout the urine. In alkaline urine the pus appears as a tenacious, gelatinous mass, which clings to the walls of the containing vessel and cannot easily be detached by shaking it; the bulk of the urine keeps permanently turbid. The distinctions above drawn, when well defined, respectively indicate that the pus in the acid urine is probably derived from the kidneys; that in the alkaline urine from the bladder. But, as with other distinctions, when ill defined they require to be interpreted with discernment. For example, a urine may be alkaline and the pus may possess a certain degree of viscosity, so that it diffuses itself through the urine in clotted masses when the containing vessel is shaken; such a condition is quite consistent with the pus being derived from an old-standing pyelitis with probably a mild form of sequential cystitis, but without any ammoniacal decomposition. Conversely, an acid reaction of the urine does not exclude the bladder as the source of the pus which may be present; an early cystitis, or one of longer standing in which ammoniacal fermentation has not been set up, often co-exists with an acid urine.

The conditions under which pus may be present in urine comprise: pyelitis, with or without sacculated kidney; in sacculated kidney, periodic flows of almost pure pus may occur, with partial or complete absence of pus from the urine during the intervals. The bursting of an abscess into the urinary passages will cause a sudden, isolated flow of purulent urine. Cystitis invariably gives rise to pyuria, but the quantity of pus may vary from that which is microscopic, to that which represents a large proportion of the bladder-contents. In chronic cystitis, the urine is usually ammoniacal from hydrolysis of the urea with the liberation of ammonium carbonate, produced chiefly by the *Micrococcus ureæ*; the ammoniacal fermentation determines the formation of crystals of triple phosphate which are present in such urines. In the acute stage of gonorrhœal urethritis more pus will be passed, during each micturition, with the first few ounces of urine than with that which follows. In the chronic stage of gonorrhœa and in gleet, shreds of agglomerated mucus, epithelium, and leucocytes may be seen floating in the recently voided, clear urine; these "gonorrhœal threads" are formed in the prostatic and urethral crypts and are periodically washed away by the stream of urine. It is said that similar filaments may be present in the urine of men who have never had gonorrhœa; this is possible, but not probable. It is important to recognise the fact that pus may appear in the urine of an apparently healthy man without

accompanying symptoms and without any ascertainable cause; in such cases the urine will probably be acid, though it may be slightly alkaline, and there will be but little epithelium present. Either spontaneously, or under treatment (in which urotropine is of great efficacy), the pyuria quickly subsides. Occasionally a small amount of pus may be due to an acute, non-specific urethritis; in this condition an early manifestation is the appearance of small, transparent, blood-stained clots of mucus, the voidance of which causes much straining. Subsequently a small quantity of pus appears, usually only for a few days; the urine is often high coloured and contains much urinary indican and urobilin. The course of this disease is very much shorter than that of gonorrhœa, from which it is further distinguished by the absence of gonococci from first to last. In women an abundant leucorrhœa, or gonorrhœa, may be the source of pus in the urine; the large quantity of squamous epithelium from the vagina that accompanies the discharge, and which may be found in the urine, affords a clue; if doubt exists, the vulva should be well cleansed and a catheter passed that has been lubricated with glycerine; if pus is present in the urine thus obtained it comes from the urinary tract.

When urine contains pus it may be important to ascertain whether the albumin that is probably present is solely due to the pus. If the pus is derived from the lower urinary passages it may give rise to little or no albuminuria; the filtrate from urines thus contaminated will not unfrequently give a negative reaction with the nitric acid test. As a general statement, it may be accepted that the greater the amount of albumin present in purulent urine the greater is the probability that it is derived from the kidneys; therefore, when the nitric acid test reveals the presence of a considerable amount of albumin it is probably of renal origin. It is to be admitted, however, that the ulcerating surface of a large vascular neoplasm of the bladder which yields pus, and at times gives rise to copious hæmorrhage may, in the intervals, exude a considerable amount of albumin without any blood colouring-matter. Purulent urine that is to be examined as to the amount of albumin it contains should be quite fresh, otherwise, by the action of bacteria, some of the albumin will have been converted into albumose. The attempt has been made to establish an albumin-pus quotient by counting the leucocytes in purulent urine with the aid of a Thoma-Zeiss hæmacytometer, and comparing the result with the amount of albumin that is present, computing it on a unit of one per cent. When the quotient is below 1:40,000 the albumin is probably due to the pus alone; when it is above 1:7000 it is probably chiefly renal. (Lint.¹)

¹ *Inaug. Dissert.*, Leiden, 1897.

CASTS.

As the name implies, casts represent the forms given to various exudative products and renal elements that are lodged for a time in the urinary tubules which thus act as matrices; the cores so formed are eventually washed away by the urine.

Casts are cylindrical bodies which vary considerably in length and in diameter; some are straight, others are convoluted. It has been assumed that convoluted casts are formed in the convoluted tubes; but it is doubtful if any consolidations there formed could reach the urine as integral casts. It is probable that the nature of the plastic material of which they are formed has no little to do with the contour of the cast: plastic substances tend to contract unequally after they become consolidated, which causes them to assume contorted, irregular shapes; in this way casts from straight tubes may become convoluted after leaving their matrices. Delépine¹ is of the opinion that the convoluted casts are formed in the narrower straight tubes, and when they arrive in the wider collecting tubes, if their progress is impeded by some obstacle, they are bent and folded on themselves; if they meet with no obstacle they remain straight. The attempt has been made to draw diagnostic inferences from the large diameter of some of the casts, and to assume that such casts indicate the participation of the collecting tubes in the inflammatory processes, and hence widespread mischief. Much stress should not be laid on differences in the size of the casts, although when taken in conjunction with the other features of the case occasional help may be obtained from comparative observations.

In a comprehensive sense the presence of casts in the urine is indicative of renal disease, but it is not the less true that, exceptionally, a few casts may be found in the absence of albumin and of any other indications of disease. Two causes may severally determine the occurrence of casts in the absence of disease: severe and sustained exercise of a very active kind, and the fugitive presence in the blood of some toxine; it is more than probable that the same ultimate factor—a toxine—is at work in both instances. The author has seen a small collection purely of hyaline casts, without any epithelium or other elements, and without any albumin, abruptly appear for a few hours in the urine of a child with purpura. In cases of jaundice casts may not unfrequently be found without any serum albumin. In some instances granular as well as hyaline casts have been seen in urine that was free from albumin.

¹ Rieder's *Atlas*, 1899.

Casts are classified according to their appearances and composition as: hyaline, epithelial, granular, fatty, and waxy. Casts chiefly formed of blood, of blood colouring-matter, and other pigments, of pus and also of bacteria occur.

Hyaline casts are (Fig. 23) exceedingly transparent, structureless bodies with most delicate outline, so much so as to be all but invisible in the microscopic field. They are of various sizes, both as regards length and breadth, and they may be either straight or convoluted. A carefully adjusted light is necessary for their detection, and it may be advisable to apply a little stain, such as gentian violet, to render them more obvious. Hyaline casts are probably formed by an exudation from the tubular epithelium, one or two cells of which may occasionally be seen embedded on the surface of the cast; when a large number of cells are so attached, the casts can no longer be described as hyaline, but as epithelial casts. Blood corpuscles may also be adherent; or, apart from the corpuscles, the hyaline substance may be permeated by hæmoglobin, which gives the cast a yellowish or reddish colour. They may also be stained by bile pigment; if this be the case, and a drop or two of very weak solution of iodine is allowed to run under the cover glass, the yellow colour of the pigment changes to green; the colourless hyaline cast is stained yellow by the same reagent.

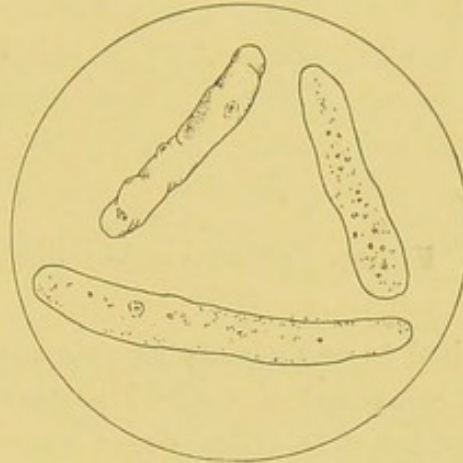


Fig. 23. Hyaline and faintly fatty casts.

Hyaline casts are found in the urine from cases of acute nephritis (early stage), chronic parenchymatous nephritis, small white kidney, granular kidney, waxy kidney, in passive renal congestion, in the last stage of diabetes mellitus, after severe and prolonged exercise and after the elimination of autogenic toxins. They have also been found after an attack of epilepsy, and in cases of acute mania.

Epithelial casts (Fig. 24) present the appearance of cylindrical collections of renal epithelium, the individual cells of which may either be well defined or they may be undergoing degenerative changes which obscure their contour. They are mostly composed of hyaline or granular cores, in which the epithelial cells are embedded; the entire core may be packed with cells or parts of it may be free; sometimes, in acute toxic nephritis, the whole of the epithelial lining of the tube comes away and in itself constitutes the cast. Whilst the hyaline cast is not necessarily indicative of renal disease, the

epithelial cast is never found except when the kidneys are undergoing inflammatory, or other pathological processes. Epithelial casts are

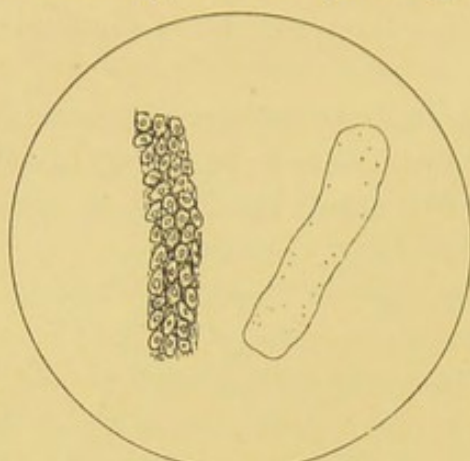


Fig. 24. Epithelial and hyaline casts.

present in the urine from cases of acute nephritis (middle and later stages), chronic parenchymatous nephritis, and occasionally in contracting kidney.

Granular casts (Fig. 25) are usually short and thick, of speckled appearance, opaque, and with boldly defined outlines. It is usual to distinguish them as finely granular and coarsely granular, but no special pathological significance is to be at-

tached to one variety as compared with the other. Granular casts are composed of proteid particles, derived—as first pointed out by Rindfleisch¹—from degenerated renal epithelium. Considerable variation in colour is one of their characteristics: they sometimes appear light grey in colour, sometimes yellow, sometimes yellowish-

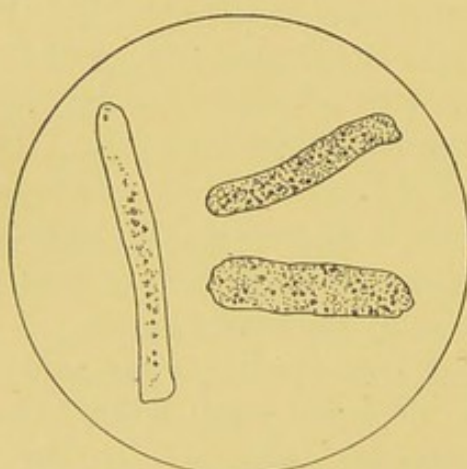


Fig. 25. Granular casts.

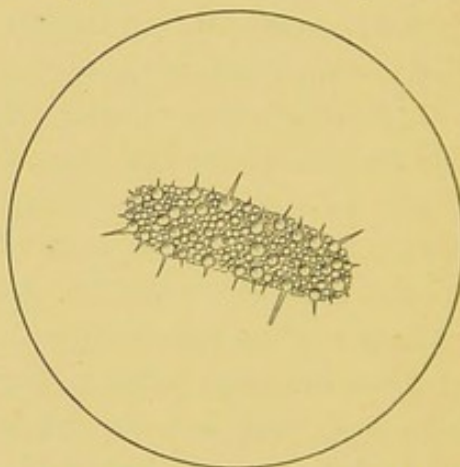


Fig. 26. Fatty cast with fat crystals.

brown, and at others reddish brown or almost black. As previously stated, the disintegrated epithelium of which granular casts are composed, may form the core of an epithelial cast; and it is not unusual to see a cast, of which one portion is epithelial whilst the rest is granular; in the same microscopic field separate granular and epithelial casts may frequently be seen.

Granular casts occur in the later stage of acute nephritis, in chronic parenchymatous nephritis, in small white kidney, and, now and then, in contracting (gouty) kidney.

¹ *Lehrbuch d. pathol. Gewebelehre*, 1869.

Fatty casts (Fig. 26) are characterised by the presence of fat-globules of various sizes arranged over the surface, or the whole cast may consist of fat-granules and globules. Usually the fatty cast has an epithelial or a granular cast for its core; the epithelial cells, or the granular matter, having undergone fatty changes, the surface of the cast is in consequence more or less closely overspread with fat-droplets. The droplets being highly refractive present the appearance of brilliant dots as they come into focus under the microscope; this is a characteristic of the fatty cast. These casts, being the result of advanced changes, are not met with in the acute stage of kidney disease, unless the degenerative processes have been very rapid, as in acute phosphorus poisoning. In the later stage of ordinary acute nephritis they may be seen, but they are most common in cases of large white kidney, and also in the small white kidney.

Occasionally tufts of needle-shaped crystals of stearic, and of some of the other higher fatty acids, probably in combination with calcium, are seen springing from the parts of the fatty casts that are most thickly covered with fat-droplets; the crystals afford further indications of advanced fatty changes in the kidneys, and are most common in cases of large white kidney. Although indicative of advanced fatty changes the occurrence of fat-crystals does not necessarily exclude the possibility of improvement in the condition of the kidneys.

Waxy casts (Fig. 27), like hyaline casts, are uniform in structure, but they have well-defined outlines, together with a tinge of pearly lustre that is quite distinctive.

They are often very broad, and, when long, are usually straight or nearly so. The contour of the cast may be interrupted by notches or fissures, such as would be produced in a fragile cylinder of feeble flexibility by bending it slightly beyond its cohesive limit; longitudinal rents may also be present. Although called "waxy" these casts are not, or are but

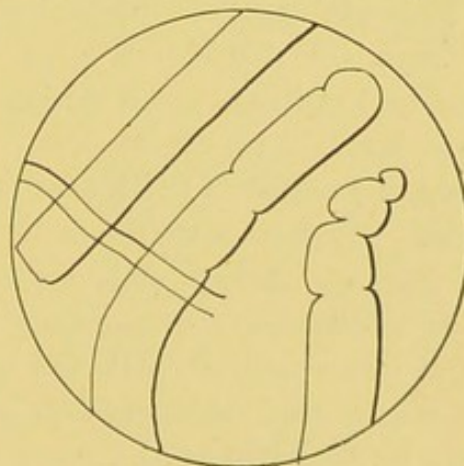


Fig. 27. Waxy casts.

rarely, composed of lardaceous or amyloid substance, and, consequently, do not give the red coloration with methyl-violet which is characteristic of tissues that have undergone amyloid degeneration. They probably consist of proteid matter which has been produced by very chronic degenerative processes affecting the tubular epithelium, and, consequently, their presence in urine is indicative of advanced chronic

disease of the kidney and of nothing more. When derived from kidneys unaffected with amyloid degeneration, the waxy cast occasionally gives the amyloid reactions—a red tint with methyl-violet and brown with aqueous solution of iodine; on the other hand, casts derived from the amyloid kidney may not yield the reaction. The waxy cast may be seen alongside granular and other casts in the urine from cases of chronic nephritis in the advanced stage; their presence forebodes evil to the patient. Fat-droplets, leucocytes, blood corpuscles, and various crystals may sometimes be seen adhering to the surface of waxy casts.

Blood casts.—The commonest kind of blood cast is formed by the coating of a hyaline or other cast with red blood corpuscles; sometimes the cast consists entirely of blood which has coagulated in the renal tubules. A third form is composed of blood pigment devoid of erythrocytes, or both pigment and corpuscles may conjointly form the cast. Blood casts are obviously indicative of the occurrence of hæmorrhage into the tubules; the pigment casts may be seen in cases of hæmoglobinuria, as well as in ordinary hæmorrhagic nephritis.

Pigment casts composed of substances other than blood pigment are recorded as having occurred; they are necessarily very exceptional.

Pus casts are rare. Like blood casts they may be composed of a hyaline core coated with leucocytes, or they may be entirely formed of pus. They occur in various suppurative diseases of the kidneys.

Bacteria casts are formed in some septic diseases implicating the kidneys. A distinction must be drawn between the true bacteria cast, and ordinary casts, or mucus-threads, which have become coated with bacteria in the bladder. Both true and false bacterial casts resist the action of acetic acid, and they readily stain with methylene-blue. The true cast differs from the bacteria-covered mucus-thread by its more uniform diameter and its limited length.

In addition to organised casts, two forms of crystalline casts may occur: in conditions attended by an excessive quantity of crystalline urates in the urine, crystals of the ammonium, or sodium biurates may be deposited in the renal tubules and be washed away in the form of veritable casts. Urate casts have been most commonly met with in the urine of infants, as a result of the uric acid infarcts which occur in early infantile life; they have also been found in gouty people. Casts of lime salts deposited in the kidney tubules are exceptionally to be seen.

False casts may consist of elongated agglomerations of urates, amorphous phosphates, crystals of uric acid, or calcium oxalate, held

together by fragments of mucus; usually they only remotely resemble tube casts, from which they may be distinguished by appropriate reagents. Another kind of false cast, sometimes called a "cylindroid" (Fig. 28), is composed of a shred of mucus which has been drawn out so that it bears a faint resemblance to a hyaline cast. It differs in being of irregularly varying diameters, often in being folded on itself in a way that reveals its flat contour, in being marked by delicate longitudinal lines, and by its (frequently) inordinate length.

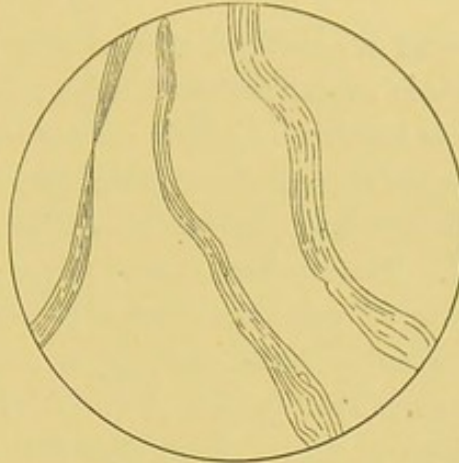


Fig. 28. Cylindroids.

Mucus-threads may be coated with various deposits, organised and unorganised, and may then be confused with granular casts; but their length and other characteristics usually render differentiation easy.

Spermatozoa may be present in the urine of males after emissions under natural conditions; they also occasionally occur after attacks of epilepsy, and sometimes in the urine first voided after an attack of apoplexy. In the course of prolonged debilitating diseases, a few spermatozoa may not unfrequently be found in the urine from time to time.

Fungi, moulds and yeasts of various kinds may appear in urine that has been exposed to the air for some time, and has thus been converted into a cultivating medium for spores suspended in the atmosphere. *Penicillium glaucum*, *Oidium*, *Torula* and *Saccharomyces* may frequently be found in saccharine urine, and also in urine which does not contain sugar.

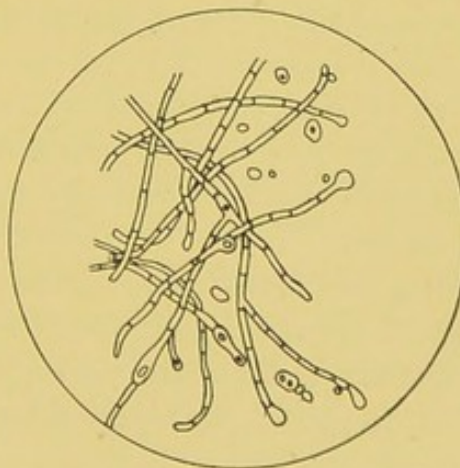


Fig. 29. Mould formed in urine.

Sarcinæ are exceptionally found in urine; they are smaller than the gastric sarcinæ from which they are developed by transmission through the urethra. The urine being an unfavourable medium for their cultivation, the sarcinæ produced in it are imperfectly formed. See Section on micro-organisms.

URINARY CALCULI.

URINARY concretions are usually composed of one or more of the inorganic constituents of urine; less frequently they are formed of organic matter. Calculi almost invariably consist of a nucleus round which are deposited concentric, or occasionally irregularly distributed, layers of the substance or substances of which the calculus is composed. The nucleus frequently consists of a minute particle of uric acid or of calcium oxalate; sometimes a semi-solid fragment of mucus or of blood-clot, or a minute foreign body, constitutes the nucleus. The initial stage of formation may take place in a uriniferous tube, or in the pelvis of the kidney, and should the calculus, whilst still small, not find its way into the bladder, it may by gradual accretion so increase in size as eventually to occupy the entire pelvis of the kidney, to which it adapts itself, and fills it like a cast. On section the structure of the calculus is seen, the layers arranged round a common centre, being usually composed of more than one substance; of 44 calculi examined by Spiegel¹ only six were found to be composed of a single substance. A renal calculus, or one that has only been a short time in the bladder, is much more likely to have a uniform chemical composition than a calculus which has been long in the bladder; a vesical calculus is usually built up of two or more constituents. Whatever may be their primal constitution, most calculi that have remained for some time in the bladder become incrustated with a coating of triple phosphates, due to ammoniacal alkalinity of the urine.

To smaller aggregations of the same substances of which calculi are formed the term "gravel" is commonly applied, a word that is erroneously used by many patients to indicate the deposit of uric acid crystals which occasionally occurs in urine shortly after it has been voided.

When sufficiently large, bisection of the calculus with the saw reveals its structure and, within limits, its composition. Its chemical constitution is definitely ascertained by reducing a fragment of the calculus to powder and then submitting it to the tests subsequently described.

URIC ACID.

About 85 per cent. of renal calculi are wholly or chiefly composed of uric acid. This calculus is brownish in colour, varying from *café au lait* to Indian red; its surface is often somewhat rough, and

¹ *Berliner klin. Wochenschr.*, 1900.

displays small projections and irregularities. The stone is hard, and is of such a texture that its cut surfaces are capable of taking a high polish. The nucleus usually has the same chemical constitution as the bulk of the calculus; occasionally the nucleus is formed of calcium oxalate, and in the larger calculi layers of calcium oxalate frequently alternate with layers of uric acid. Small calculi composed of urates are occasionally met with; they are soft and are yellowish in colour, and consist either of ammonium or of sodium biurate.

Identification.—A fragment of a uric acid or of a urate calculus treated in a porcelain capsule with a few drops of nitric acid and then heated on the water-bath to dryness leaves a deep, orange-coloured product (alloxantin); when the capsule is cold, the addition of a drop or two of ammonia changes the colour to lake or purple (ammonium purpurate), which on the subsequent addition of a little solution of soda becomes more blue (sodium purpurate). If the calculus consists of ammonium biurate it may be dissolved, with the aid of heat, in dilute hydrochloric acid; on the subsequent addition of a fixed alkali ammonia vapour is evolved, and may be detected by holding immediately over the solution (but without touching it or the walls of the containing vessel) a piece of moistened red litmus-paper, which becomes blue. Or a glass rod that has been dipped in strong hydrochloric acid may be held close above the surface of the solution; this produces smoke-like fumes of ammonium chloride. Urate concretions are soluble in hot water.

CALCIUM OXALATE.

This calculus, which is known as the mulberry calculus, occurs next in frequency to the uric acid calculus. It is usually dark-brown or greyish in colour. When small and still in the kidney, or only recently descended into the bladder, it is smooth and probably lightish in colour; as it increases in size it becomes rough, irregular, and nodulated, and darker in colour, whence the name mulberry calculus. It is very hard and is difficult to crush. On section the larger calculi are frequently found to have a nucleus of uric acid, and to be composed of alternate layers of uric acid and calcium oxalate; a calculus entirely composed of calcium oxalate is much less common. The oxalate calculus is only formed in acid urine.

Identification.—Calcium oxalate is insoluble in acetic acid (distinction from calcium phosphate), but is soluble without effervescence in strong hydrochloric acid. If to the solution thus obtained, excess of a solution of sodium acetate is added, calcium oxalate is again

formed and thrown down. Some of this precipitate, or of the original calculus, after being ignited with the blowpipe, turns red litmus-paper blue, and dissolves with effervescence in acetic acid; the addition of ammonium oxalate to the solution produces a precipitate which proves the presence of calcium.

CALCIUM PHOSPHATE.

Very exceptionally, calculi are formed of calcium monohydric phosphate; these calculi are almost always homogeneous, *i.e.*, they consist of calcium phosphate only; now and then, calculi composed of alternate layers of calcium phosphate and some other substance, such as uric acid, are met with. The homogeneous calculus is white, smooth, and moderately hard. It is to be distinguished from calculi that are surrounded with a secondary deposit of triple phosphates; such deposition takes place in the bladder, after the occurrence of the alkaline fermentation which furnishes the ammonia of the deposit. Calculi entirely composed of triple phosphates, sometimes called fusible calculi, are not often met with; on the other hand, the secondary deposition of triple phosphates is very common, and may be so abundant as to constitute the main bulk of a calculus essentially composed of uric acid, calcium oxalate, or other substance. The incrustation of triple phosphates is white and friable, and is often unsymmetrically deposited.

Identification.—Calcium phosphate is infusible in the blowpipe flame. If a portion of a calcium phosphate calculus is dissolved in hydrochloric acid, the addition of a little solution of ammonium molybdate produces a yellow precipitate which is soluble in ammonia.

Triple phosphate fuses in the blowpipe flame, hence the term "fusible earth." Both the calcium phosphate and the triple phosphate are soluble in acetic acid.

CALCIUM CARBONATE.

Calculi are rarely formed of this substance; those which have been found were mostly small in size. They are white, or yellowish-white, in colour and are not easily crushed. Spiegel¹ found calcium carbonate to be present in calculi chiefly composed of uric acid, urates, and xanthin; that is to say, in concretions formed in acid urine. Calcium carbonate is not infrequently deposited on calculi of other composition.

¹ *Loc. cit.*

Identification.—Calculi of calcium carbonate dissolve with effervescence in hydrochloric acid, and the solution thus obtained gives a precipitate with ammonium oxalate.

CYSTIN.

In cases of cystinuria, attention is often first directed to the condition by the occurrence in the urine of cystin in the solid state, either as a sediment or as gravel; in some instances, which are of rare occurrence, a calculus is formed. The cystin calculus, yellow in colour, is somewhat translucent, and its surface has a crystalline appearance, which is very obvious when examined through a lens. These characteristics, together with a somewhat soft and friable structure, make the cystin calculus easy of recognition. It is usually homogeneous, though, occasionally, cystin is deposited round a nucleus of foreign composition. When a cystin calculus has been exposed for a long time to the action of light, the exposed surface turns green, about the same tint as crystalline ferrous sulphate.

Identification.—When heated on platinum foil in the Bunsen burner cystin burns with a smoky flame, and gives off the odour of sulphurous acid without leaving any residue. A fragment of the calculus may be dissolved in ammonia, and, on exposure to the air, the solution presently deposits hexagonal crystals of cystin. From ammoniacal solution cystin is precipitated by acetic acid.

XANTHIN.

Calculi formed of this substance are of the rarest occurrence. They are brownish-yellow or honey-coloured, and are moderately hard with a smooth surface; they are usually homogeneous in composition. Lebon¹ records an instance to the contrary: externally, the calculus displayed layers of calcium phosphate, triple phosphate and calcium oxalate; the bulk of the calculus consisted of xanthin mixed with uric acid.

Identification.—Xanthin, like cystin, is readily soluble in ammonia; on the addition of a solution of silver nitrate to the ammoniacal solution a copious precipitate of silver-xanthin is thrown down. Xanthin does not give the murexid reaction; but, by substituting chlorine-water for nitric acid, a very similar reaction is obtained (see Xanthin, page 148).

Isolated instances of concretions of **cholesterin** have been recorded. Horbaczewski² analysed a calculus which weighed over

¹ *Compt. rendus*, 1871.

² *Zeitschr. f. physiol. Chem.*, 1893.

360 grains and found that it consisted of 95.84 per cent. of cholesterolin. At the necropsy on the body of a woman, Glinski¹ found five irregularly formed stones in a calyx of one of the kidneys, along with one in the ureter, all of cholesterolin. During the last month of her life, the patient had passed a large quantity of cholesterolin crystals in the urine.

Fatty concretions, of doubtful origin, have also been met with.

¹ *Wratsch*, 1893.

URINE IN ITS PATHOLOGICAL RELATIONS.

OLIGURIA.

THE pathological conditions which are accompanied by diminished excretion of urine are : cardiac dilatation, and failure of compensation in all forms of heart disease ; retention of water in the system, either generally as in febrile states, or locally as in dropsy, including hydrothorax ; acute nephritis, the blocking of the ureter by a calculus, or by kinking due to the abnormal position of a movable kidney ; diarrhoea and cholera, and persistent vomiting, with or without dilatation of the stomach. Reflex anuria may follow the introduction of a sound into the bladder ; very rarely it may occur after labour. Bacialli and Collina¹ report a case in which the injection of very hot water into the vagina caused reflex anuria for six days, when death took place from the local injuries the patient had sustained, without the occurrence of any uræmic symptom. Rénou² saw a case of anuria which lasted seven days without uræmia ; still longer intervals of suppression of urine without uræmia are reported, but with doubtful authenticity. To a condition of delayed excretion of the urine, the name "opsiuria" has been given. (Gilbert and Lereboullet³.) It has been found to occur in some hepatic diseases—biliary cirrhosis and cardiac-liver, and also in enlarged spleen ; it is supposed to be due to plethora of the portal system, which delays the absorption of water from the intestines.

When the quantity of the urine is much below the normal, attempts are usually made to increase it by the administration of diuretic drugs, which act in several ways. Saline drugs, such as ammonium and potassium acetate, are supposed to act by withdrawing fluids from the tissues, an action that has been attributed to an alteration in the osmotic pressure ; probably urea, which has been found to possess diuretic properties when administered by the mouth, acts in the same way.

¹ *La Clinica med. Ital.*, 1900.

² *Bull. des Hôpitaux*, 1900.

³ *Compt. rend. Soc. Biol.*, 1901.

The diuresis produced by the intravenous injection of saline and other solutions is to be accounted for on other grounds : to a limited extent and for a short time the blood pressure is increased, the heart-beats are quickened, and the volume of the kidneys is enlarged on account of vascular dilatation, whilst the molecular concentration of the blood is but little, if at all, altered. Thompson¹ found that the intravenous injection of 0.6 to 0.9 per cent. solutions of sodium chloride produces diuresis in dogs, although the specific gravity of the blood is not altered ; the diuresis is accompanied by an increase in the excretion of the urea and of the total nitrogen, but not of sodium chloride. Thompson does not believe that the diuresis is caused by elevation of the blood-pressure, as it fell during the greatest secretion of urine ; the hydræmic condition of the blood plays an important rôle, but it is not the sole factor ; he sees no parallelism between the kidney-volume and the urinary outflow. Starling² regards the diuresis produced by the intravenous injections of crystalloid substances as due to two factors : (1) hydræmic plethora with consequent rise in the blood-pressure and velocity of the blood in the kidneys ; (2) a direct dilator effect produced by the injected substances on the renal blood-vessels. Starling considers the glomerular epithelium to be a simple filtering membrane, and finds a complete parallelism between the volume of the kidneys and the secretion of the urine. Hédon³ also found that the intravenous injection of hypertonic solutions of glucose, in the first instance causes diuresis by increasing the blood pressure, and subsequently by dilating the renal blood-vessels. Balthazard⁴ is of the opinion that the intravenous injection of hypertonic solutions increases the flow of water, but that it deranges the normal output of the solid urinary constituents and endangers the stability of the red blood-corpuscles ; for clinical purposes, therefore, he recommends subcutaneous instead of intravenous injections of hypertonic solutions, as they are equally efficient in promoting diuresis and at the same time are free from danger.

When low blood-pressure is the cause of oliguria, it is a common clinical experience that the administration of cardiac tonics, such as digitalis, *especially if aided by rest*, is followed by diuresis, due to increased vascular tension. Diuretics of another type, the methylpurins, of which theobromine is the most powerful, have a wider range of action : they invigorate the heart, dilate the blood-vessels, and stimulate the epithelium of the convoluted tubules of the kidneys. Anten⁵ states that whereas saline diuretics increase the output of

¹ *Journ. of Physiol.*, 1899.

² *Ibid.*

³ *Compt. rend. Soc. Biol.*, 1900.

⁴ *Ibid.*

⁵ *Arch. internat. d. pharm. et d. thérap.*, 1901.

water and salts, the xanthin bodies also increase the output of nitrogen in the form of urea and uric acid.

An entirely different type of diuretic agency has been experimented with by Denoyés and others.¹ Three young men were submitted to the action of high-frequency currents for from six to twenty-five minutes daily, from three to seven days, with the result that the quantity of urine, together with the percentages of urea, uric acid, phosphates, sulphates, and chlorides were increased, as was also the relation of urea to total nitrogen. It is difficult to account for such results, even in the healthy subject; and it is more than doubtful that any benefit could be derived from the use of electricity in pathological oliguria.

POLYURIA.

Apart from those diseases in which, in addition to inordinate amount, the urine is abnormally constituted, an excess of very dilute but otherwise almost normal urine is met with in two forms—simple polyuria and diabetes insipidus.

Simple polyuria is not an uncommon condition; it is most frequently met with in hysterical girls and women. In addition to the copious diuresis which succeeds an "hysterical attack," cases of functional neuroses not infrequently occur in which the patient for weeks or months passes three or four times the usual quantity of urine, limpid and of low specific gravity. Less frequently the patient is an emotional young man. The distinction between this type of polyuria and that about to be described is one of degree: the daily amount of urine is less, and with it the thirst; the emotional state and the prevailing sex of the patients serve as diagnostic indications.

DIABETES INSIPIDUS.

Diabetes insipidus is a rare disease, much rarer than diabetes mellitus. It occurs more frequently in males than in females in the proportion of nearly 3 : 1, and usually between the ages of twenty and forty years, though it has been seen in an infant, two months old, that passed from thirty-five to upwards of fifty ounces of urine, s.g. 1001, in the twenty-four hours (Cozzolino²); it has also occurred at the age of seventy years. Amongst the predisposing conditions are: heredity, trauma, pregnancy, cerebral disease, alcoholic excess, and fright, prolonged anxiety, or other severe mental disturbance. Usually the first symptom to attract attention

¹ *Compt. rendus*, 1901.

² *Policlinico*, 1900.

is inordinate thirst; then follows excessive diuresis, the quantity of urine often exceeding that excreted in diabetes mellitus, amounting to from twenty to forty pints daily. It has been stated that the quantity of urine excreted in the twenty-four hours may exceed that of the liquids imbibed; this can only occur for a limited time, until the tissues have been partially desiccated; when this has taken place the output of urine may nearly balance, but it will not exceed, the intake of liquids, including of course any that may be present in the solid food that has been eaten. In many diabetics there is an unaccountable tendency to conceal from the knowledge of their attendants the full extent of the intolerable thirst that torments them; to accomplish this they secretly supplement the measured supply, although no limit has been imposed. The urine is limpid and almost colourless; its reaction is acid, and its specific gravity is low, from 1001 to 1005, though sometimes it is higher. The percentage of urea is low, but the daily amount is brought up to, or even beyond, the normal by the excessive diuresis; as much as 80 grms. have been excreted (Gerhardt¹).

According to v. Jaksch,² the nitrogen ranges from 0.14 to 0.18 per cent. The daily output is very variable, amounting to from 9.30 to 16.90 grms., of which the amido acids may furnish from 5.4 to 8.2 grms.; the amido-nitrogen may amount to 49.40 per cent. whilst the urea-nitrogen only reaches 47.70 per cent.

The daily excretion of uric, sulphuric, and phosphoric acids is not materially altered; sometimes the phosphoric acid is greatly in excess, constituting what has been called phosphatic diabetes. The chlorides are usually increased. Inosite has frequently been found in the urine, owing to the inordinate flushing of the tissues by the large amount of water that passes through them. Creatinin is sometimes present in great excess; at others it is much diminished (Tedeschi³). Small quantities of pentose were found by Alfthan.⁴

In diabetes insipidus the kidneys are not necessarily affected, although after a time they may show indications of structural changes. The condition is due to a disorder of the nerve-centres which causes a rapid and increased passage of blood through the kidneys, so that it parts with an excessive amount of water. In some instances, diabetes insipidus appears to be primarily due to polydipsia; usually the diuresis is the cause of the polydipsia. Exceptionally, traces of sugar have been found in the urine, and occasionally the disease merges into diabetes mellitus, or conversely, diabetes mellitus into diabetes insipidus, which is less infrequent.

¹ *Spec. Path. u. Therap.*, 1899.

² *Zeitschr. f. klin. Med.*, 1902.

³ *Riv. Veneta d. Sc. Med.*, 1901.

⁴ *Berliner klin. Wochenschr.*, 1902.

Sometimes traces of albumin are met with in the urine from cases of diabetes insipidus.

DIABETES MELLITUS.

In clinical practice three types of pathological glycosuria are met with : *Acute diabetes*. *Chronic diabetes*. *Simple glycosuria*.

Acute diabetes is most commonly met with in the early and middle periods of adult life ; it occurs more frequently in males than in females. It is characterised by the classical signs of diabetes : thirst, polyuria, increased appetite, with progressive emaciation and debility. The urine is copious, usually exceeding 100 ounces, and possibly reaching twenty or thirty pints in the twenty-four hours. It is limpid and pale, with a faint yellow or greenish-yellow tint. The specific gravity ranges from 1030 to 1040 ; it may be higher, but very rarely exceeds 1050.

The amount of sugar is usually below 3 or 4 per cent. ; very rarely it has been known to reach 10 per cent. ; the daily excretion ranges from a few ounces up to two or more pounds. The reaction of the urine is usually acid ; it may be excessively so. Little, if any, mucous cloud is visible, but as the sugar affords a medium for the growth of *Torula*, diabetic urine rapidly becomes turbid, especially in warm weather. The daily excretion of urea is largely increased ; it may reach four or more times the normal amount. In very severe cases in which there is an enormous excess of ammonia, the urea-nitrogen will be proportionately diminished. The amount of amido-nitrogen may be increased to 0.64 grm. in the twenty-four hours (v. Jaksch).¹ Excess of uric acid has also been observed ; more commonly it remains unaltered. Occasionally, crystals of uric acid are deposited which differ in appearance from the uric acid crystals commonly seen in urine, inasmuch as they are colourless, or nearly so ; exceptionally, they have a canary-yellow tint. The chlorides and total sulphur are considerably increased and, in severe cases, the phosphoric acid also. Excess of ammonia and lime is of grave significance as indicating acidosis ; the latter especially being derived from the osseous system discloses an urgent demand for bases to combine with and to neutralise an inordinate excess of acid products. Ammonia may be present in four or five times the normal amount, and calcium has been found in upwards of ten times the normal amount. In mild cases, neither the phosphoric acid, the ammonia, nor the lime exceeds the normal proportions. In the pre-comatose period, Herter² found that the ammonia-nitrogen amounted to from 16 to 30 per cent. of the total

¹ *Zeitschr. f. klin. Med.*, 1903.

² *Journ. Experiment. Med.*, 1901.

nitrogen; he recommends that the amount of organic acids in the urine of diabetes should be determined at least once a month. Acetone is frequently present in considerable quantity, and diacetic acid also; the latter is of graver import, as pointing to the presence of β -oxybutyric acid and consequently to the peril of acidosis and coma. It is to be noted, however, that it is not unusual for diacetic acid to appear for shorter or longer periods without any indication being afforded other than the chemical reaction of the urine. Excess of oxalic acid in diabetic urine is of doubtful occurrence, and when it does occur it is probably directly due to the food; in mild cases no excess occurs. Albumin is not often present in the urine of acute diabetes unless in the advanced stage, and then but in small amount; in some cases (the patients frequently being of a stout build) albuminuria of a pronounced type may be present from an early period. Occasionally diabetes merges into Bright's disease; the sugar disappears and albumin takes its place. Külz¹ directs attention to the appearance of a large number of hyaline and finely granular casts in the comatose stage of diabetes; in fifteen out of sixteen cases of diabetic coma, Williamson² found an enormous quantity of finely granular casts, but only either immediately before, or actually during the state of coma.

Chronic diabetes and **simple glycosuria** are most common at, or after, middle life. The symptoms of chronic diabetes are much less pronounced than those of acute diabetes: there may be but little thirst and the loss of weight is very gradual, the patient looking flabby long before there is any obvious emaciation. The urine is probably not excessive in quantity; it is usually limited to from two to five pints a day. It is often high coloured rather than pale; its specific gravity rarely exceeds 1030, and it may be as low as 1010. The amount of sugar is small, ranging from less than half per cent. up to 2 or 3 per cent.; the daily amount excreted does not exceed 200 or 300 grains. The reaction of the urine is acid. In some cases there is an excessive percentage of uric acid and of urea; an increase in creatinin has also been observed. Not infrequently a large excess of indoxyl is present. A great number of cases of chronic diabetes are essentially alimentary: the patients are stout, of florid complexion, and probably have a gouty history; they display the tokens of "good living" along with insufficient outdoor exercise. Notwithstanding its high colour, the urine of such patients has a tendency to deposit crystals of uric acid shortly after it is voided.

Simple glycosuria is solely indicated by the presence of sugar in the urine, without any other symptom suggestive of diabetes; though

¹ *Lyon Médicale*, 1892.

² *Diabetes Mellitus*, 1898.

most frequent after middle life, it occasionally occurs in young people. In this condition, which is usually of limited duration, the presence of sugar in the urine is frequently discovered accidentally, or as the result of methodic examination of the urine of all patients, since no complaint is made of suspicious symptoms. It is to be borne in mind that sugar may be present in urine of low specific gravity; over one per cent. has been found with a specific gravity of only 1007.

Chronic diabetes and simple glycosuria are characterised by the pronounced effect produced on the urine by appropriate dietetic restrictions. In both (more especially in simple glycosuria) it is comparatively easy to arrest, or to check the excretion of sugar by withdrawal of the carbohydrates; whilst in acute diabetes the most rigid dietary often fails.

ACUTE FEBRILE DISEASES.

In most febrile diseases the urine is of higher specific gravity, of deeper colour, often like that of strong ale, and less in amount than in the healthy state; the alteration in colour is partly the result of concentration, but it is chiefly due to excess of urobilin. The oliguria of the febrile state is to be accounted for, to some extent, by retention of water in the tissues; after the crisis of the fever diuresis often occurs. When voided, febrile urine may be clear and bright and may remain so for a while; if tested with nitric acid for albumin such urine will often at once develop a urate cloud, and in a few minutes a deposit of urea nitrate crystals will form on the surface of the acid. In the later stages of the febrile state, spontaneous deposition of urates takes place and is regarded as a sign of the crisis in the fever, especially in pneumonia. The reaction of the urine is freely acid. Pick¹ states that while the urine is strongly acid during the febrile period, it almost always becomes either amphoteric, or even alkaline, after the crisis; he attributes this to the setting free of the soda from the exudation-products, and from the tissues generally, where it has been retained. In severe febrile conditions, in which the tissues are rapidly metabolised, the normal proportion of potash and soda excreted in the urine may be inverted, the amount of potash being greater than that of the soda. Chloride-retention also occurs, especially in pneumonia in which there may be entire absence of chlorides during the acute stage; about twenty-four hours after the crisis they begin to re-appear, but do not reach the average normal amount for a week or ten days. In scarlet-fever there may be no retention of chlorides. (Hutchison².) The

¹ *Arch. f. klin. Med.*, 1900. ² *Journ. of Pathol. and Bacteriol.*, 1898.

phosphates may be diminished or increased; in some febrile diseases, as in malaria, they are almost absent. The sulphates have been found to be increased in enterica and pneumonia. The urea and the uric acid are usually increased in the acute stage of the fever; somewhat later an increase has been observed in the excretion of creatinin (Senator¹), especially in enterica, along with excess of ammonia. In febrile diseases generally, Tedeschi² found that the increase in creatinin runs parallel with the tissue waste. In typhoid fever, v. Jaksch³ found that the urea-nitrogen may be diminished, the loss to some extent being compensated by a considerable excess of bodies of the amido-acid type, which may represent from 30 to 35 per cent. of the total nitrogen. In most cases of pneumonia, Cook⁴ states that more nitrogen is excreted during resolution than the exudation-products would account for. In rapid resolution, the leucocytosis-curve closely follows that of the nitrogen excretion, which would seem to point to a causal relation between leucocytosis and resolution.

As might be inferred, the metabolism that occurs in the course of a prolonged and severe febrile attack does not produce a uniform array of excretory products in the urine; variations occur not only in relation to the different febrile diseases, but also in the successive stages of the same disease. An indication of the variations which occur in the febrile state is given by Moraczewski,⁵ who found that in the first stage of fever the elevation of temperature is followed by increased excretion of chlorides and diminished excretion of urea and of phosphoric acid; during the continuation of the fever the excretion of chlorides and phosphoric acid falls and that of the nitrogen rises; subsequently the excretion of phosphoric acid increases, and at this stage the urine acquires the typical febrile characteristics: the chlorides are below, and the nitrogen and phosphoric acid are above, the normal. When the temperature subsides the retention of the chlorides for a time is still greater and the nitrogen and phosphoric acid are more freely excreted; then the excretion of the chlorides progressively increases to the normal level, to which the nitrogen and phosphoric acid subside.

In all febrile conditions in which the pyrexia is high a faint trace of albumin—febrile albuminuria—may be present in the urine; in some, notably in diphtheria and scarlet fever, albuminuria of another type may occur. In about 50 per cent. of cases of diphtheria a considerably larger amount of albumin is present in the urine than

¹ Virchow's *Arch.*, 1877.

² *Riv. Veneta di Scienze Med.*, 1901.

³ *Zeitschr. f. klin. Med.*, 1902.

⁴ *Johns Hopkins Hospital Bulletin*, 1902.

⁵ Virchow's *Arch.*, 1899.

the febrile state will account for; it appears early in the course of the disease; it is not due to nephritis; and it usually subsides during convalescence. In scarlet fever febrile albuminuria may occur, without being of special significance; a larger amount of albumin, which may occur later on, after the pyrexia has subsided, has great significance as being indicative of the probable onset of nephritis.

Some febrile conditions of an infectious nature may be accompanied by the presence of specially associated products in the urine, which, when recognised, may serve as aids to diagnosis. Giarre¹ and Binet² found very little urobilin in diphtheria, but great excess in scarlet fever, and most of all in pneumonia. Traces of acetone are present in the urine in many febrile conditions, and in scarlet fever, follicular tonsillitis, and quinsy there may be much more than a trace, whilst in diphtheria it is often all but absent. Phenol and indoxyl compounds are present in excess in many febrile conditions, especially in tuberculous infection of the intestinal tract; in typhoid the amount varies, but there is usually an increase in the phenol; Blumenthal³ states that excessive indicanuria in the febrile stage of enterica indicates a severe attack. When intestinal hæmorrhage occurs in enterica, or in other diseases, a large increase in urobilin and the aromatic compounds appears in the urine. Garrod, Kanthack, and Drysdale⁴ examined the urine in three cases of enterica at the time the patients were voiding green stools, probably due to unchanged biliverdin; urobilin was absent, or nearly so, whilst urochrome, uroerythrin, and hæmatoporphyrin were found to be present. In febrile urine a perceptible increase in hæmatoporphyrin is not infrequently present—in acute rheumatism, gout, enterica, and pneumonia for example. Uroerythrin is usually increased in febrile diseases, but not much in enterica. Many observers have reported the occurrence of albumose in the urine in febrile diseases, especially in enterica, pneumonia, and scarlet fever; it is to be remembered, however, that the three diseases named are distinguished by the presence of a large amount of urobilin in the urine and, as previously pointed out (*cf.* tests for albumoses), the biuret-reaction is frequently held to prove the presence of an albumose notwithstanding the fact that urobilin yields the same reaction. In acute febrile conditions, which are accompanied by the formation of collections of septic pus, such as quinsy and empyema, a considerable amount of volatile fatty acids has been found in the urine. Glycerinphosphoric acid has been found in some febrile conditions, and in advanced phthisis.

¹ *Lo sperimentale*, 1895.

² *Revue Méd. de la Suisse rom.*, 1894.

³ *Loc. cit.*

⁴ *St. Bartholomew's Hosp. Reps.*, 1900.

GOUT.

In **acute gout**, the urine, reduced in amount, is concentrated, high-coloured, of moderately high specific gravity, 1018-1025, either clear or clouded with urates, and occasionally there is a deposit of uric acid. There may be a little albumin. Excess of urobilin and uroerythrin usually occurs, and also occasionally of hæmatoporphyrin.

In **chronic gout** the urine, plentiful in amount, is lightish-coloured, clear, with a specific gravity of 1005-1018, and frequently with a trace, or more, of albumin, and possibly of a few granular or hyaline casts. Luff¹ considers that permanent albuminuria is a fairly common occurrence in confirmed gout. Duckworth² has observed a high specific gravity of the urine in many members of families of gouty proclivity.

The innumerable experimental investigations concerning the nature of gout, a disease which is supposed to result from a specific form of abnormal metabolism involving processes that directly appeal to chemistry for their elucidation, have been singularly unproductive of practical issue so far as any useful knowledge of resultant changes in the urine goes. Attention has been focused on uric acid since Garrod discovered it in the blood of gouty patients, and for long the spontaneous deposition of crystals of uric acid from urine was held to afford ample proof of its presence in excess; and further, inasmuch as attacks of acute gout are accompanied by the deposition in certain tissues of uric acid in combination with sodium, a coincident diminution of the urinary uric acid was assumed as a corollary. Neither of these assumptions is any longer tenable, as recent researches conducted with improved methods show that little if any change occurs in the output of uric acid during an attack of acute gout. Badt,³ Magnus-Levy,⁴ and His⁵ found an increase rather than a decrease. His found, from one to three days before an acute attack, that the output of uric acid was diminished; after the attack an increase took place which reached its maximum in from one to five days; he further found that during the attacks, and during the intervals, the average daily excretion in gouty subjects showed no characteristic differences, nor does the average daily excretion of uric acid in gouty patients differ from that of healthy people. Magnus-Levy found the increase to occur on the first day of the attack and with every important accession of the pain.

¹ *Gout*, 1898.

³ *Zeitschr. f. klin. Med.*, 1899.

⁵ *Deutsch. Arch. f. klin. Med.*, 1899.

² *A Treatise on Gout*, 1889.

⁴ *Ibid.* 1898.

It has been observed that considerable nitrogen-retention occurs in gout; in chronic gout this has frequently been known to amount to several grammes daily; on some occasions Vogel found it to reach from 2 to 4 grms. Luff finds the amount of uric acid eliminated in chronic gout to be diminished. Nitrogen-retention also occurs in acute gout. Vogt¹ investigated the excretions of a patient for two weeks after his first attack of gout, alongside those of a healthy man of similar build. Both lived on the same diet, the amount of nitrogen and phosphoric acid it contained being ascertained by analysis. The observations were divided into three periods: one of six days and two succeeding periods of five days each. During the middle period both the men ate 175 grms. of thymus in addition to the diet they took in the first and last periods. The final results showed that the nitrogen-balance of the gouty patient, who had retained 24 gm. of nitrogen, but no P_2O_5 , was always more favourable than that of the healthy man; so that nitrogen-retention occurs in acute as well as in chronic gout. Waldvogel² investigated the metabolism during an attack of acute gout from the first to the eleventh day, and also two days previous to the succeeding attack. From the beginning of the attack the output of uric acid was increased, and on the third day it reached a maximum of 0.824 gm., at which point it remained for seven days and then sank until the next attack. The nitrogen excretion and also that of phosphoric acid was reduced. In the early period of an attack of gout, Watson³ observed diminution in P_2O_5 with subsequent increase. There was excess of uric acid which continued to increase during the first three days. The total nitrogen was increased, especially in the later period of the attack. No disturbance of the ratio of uric acid to urea occurred. Schmoll⁴ found considerable retention of nitrogen with probably no retention of uric acid; the alloxur bodies were not increased and their relation to uric acid was normal. In one case Galdi⁵ found the daily excretion of uric acid to be 1.452 grms. and of xanthin bases 0.070 gm.; in another case the uric acid was only 1.020 grms., whilst the xanthin bases reached 0.111 gm. Comparatively only a few investigations have been made with regard to the amount of the xanthin bases in the urine of gouty patients during and between attacks; Kaufmann and Mohr⁶ found them frequently to be of normal amount in chronic gout; further investigations in this direction are needed. The phosphoric acid excretion

¹ *Deutsch. Arch. f. klin. Med.*, 1901.

² *Centralbl. f. Stoffwechsel u. Verdauungskrankh.*, 1902.

³ *Brit. Med. Journ.*, 1900.

⁴ *Zeitschr. f. klin. Med.*, 1896.

⁵ *Arch. f. exp. Pathol.*, 1903.

⁶ *Deutsches Arch. f. klin. Med.*, 1902.

is usually increased during the attack; occasionally it may be diminished. Schmoll considers that it runs parallel with the nitrogen excretion. Croftan¹ approaches the uric acid question in another way. Starting with the assumption that the tissues possess the power of destroying uric acid, he experimented with aqueous extracts obtained from organs removed from the human body in order to ascertain their relative uric acid-destroying activity. He found that the kidneys destroy more uric acid than any other organ. Whilst regarding the accumulation of uric acid as only one of the symptoms of gout, and not as a cause, Croftan considers that renal insufficiency is an important factor in that disease; his view of insufficiency being want of destroying power; the older view being want of eliminating power. He infers that the kidneys (and other organs) secrete unorganised soluble ferments which possess the power of destroying uric acid. Soetbeer² examined the urine of a patient with gout, alongside that of two healthy persons, all three being on the same diet during the investigation and for three days previously. In the gouty urine, the sodium, magnesium and phosphoric acid were unaltered; the sulphuric acid and the nitrogen were slightly reduced. The potassium, calcium and ammonia, and also the uric acid, were much less than in the control urines. The gouty urine was more acid: in it the acids exceeded the bases by 0.44 grm.; whilst in the control urines, the bases (reckoned as soda) exceeded the acids by from 0.6 to 0.9 grm.

Indoxyl is sometimes present in the urine in considerable excess, probably from intestinal putrefaction, the digestive processes being much deranged during an attack of gout. Glycosuria is not infrequently met with; usually the amount of sugar is small.

DISEASES OF THE BLOOD.

In the course of the various anæmias, with certain exceptions, the changes that occur in the urine are less marked than might be expected. In **simple anæmia** the urine is pale, feebly acid, or faintly alkaline, and of low specific gravity. The daily output of nitrogen is little altered, showing that the proteid metabolism is not materially affected; very severe anæmia constitutes an exception to this statement. Now and then a considerably increased excretion of nitrogen has occurred, more than double the average normal; in some cases, in which the elementary composition of the food was determined, a negative N-balance was observed to occur for a time, followed by

¹ *N. York Med. Record*, 1903.

² *Zeitschr. f. physiol. Chem.*, 1903.

N-retention. The same irregularity may occur in the course of the phosphoric acid excretion. Hale White and Hopkins,¹ in a case of anæmia, found the ratio of $P_2O_5:N$ almost exactly as in the normal individual. The excretion of uric acid also is very irregular: an increase is found in one case, and a decrease in another.

In leucocythæmia, on the other hand, certain definite changes are frequently observed, especially as regards the excretion of uric acid, which is usually found to be much increased; not infrequently it is twice or thrice the normal average, and in exceptional instances it has been found to reach three and even five grammes in the twenty-four hours. An increase has also been observed in the other urinary purins. Stejskal and Erben² state that the uric acid excretion is less in lymphatic than in splenic leucocythæmia, whilst the contrary holds good for the xanthin bases. Magnus-Levy³ thus differentiates the changes in metabolism which occur in acute and in chronic leucocythæmia: in the acute form there is loss of nitrogen, which may be very excessive, reaching 21 grms. daily, or more. A large amount of uric acid may also be excreted, the increase, however, fails to coincide exclusively with the increase in leucocytes; this is corroborated by the observations of Hale White and Hopkins. In chronic leucocythæmia, Magnus-Levy finds no increase in the output of uric acid, nor in that of the total nitrogen. It appears that the relation between increase of leucocytes and excess of uric acid excretion is not absolute and essential. A patient with leucocythæmia was kept on purin-free diet for a fortnight; during this period the uric acid excretion ranged between 0.77 and 1.98 grms. daily, giving an average of 1.36 grms., and yet the leucocytes did not exceed 30,000 in the cubic millimetre (Van der Wey).⁴ In a case in which the leucocytes were reduced to 1500 to 3000 in the cubic millimetre, Hutchison and Macleod⁵ found no distinct diminution in the alloxur bodies and P_2O_5 , the patient being on an alloxur-free diet; the leucopenia was probably due to increased destruction of leucocytes, rather than to diminished activity of the bone marrow. In cases of medullary leucocythæmia, v. Jaksch⁶ observed an increase in the amido acid-nitrogen at the expense of the urea nitrogen; on the other hand, Halpern⁷ states that the distribution of the nitrogenous bodies of the urine is perfectly normal; that there is neither excess nor diminution in the amido acid-nitrogen. The creatinin has been found to be reduced to less than one-half the

¹ *Journ. of Physiol.*, 1900.

³ *Virchow's Arch.*, 1898.

⁵ *Journ. of Exp. Med.*, 1901.

⁷ *Ibid.* 1903.

² *Zeitschr. f. klin. Med.*, 1899.

⁴ *Deutsches Arch. f. klin. Med.*, 1896.

⁶ *Zeitschr. f. klin. Med.*, 1902.

normal. Phosphoric acid retention has been observed in leucocythæmia as much as 50 per cent. of the amount taken, according to Moraczewski¹; by Blumenthal it was found to be normal. In spleno-medullary leucocythæmia, Milroy and Malcolm² observed retention; and Hale White and Hopkins³ observed the same in splenic leucocythæmia. In lymphatic leucocythæmia, Stejskal and Erben, found retention of N and Cl, and in less degree of P_2O_5 , along with loss of CaO; in spleno-medullary leucocythæmia the N-balance was maintained. Bartoletti⁴ found a diminished amount of iron in the urine of leucocythæmic patients.

In **pernicious anæmia** the urine is dark-coloured; it is usually clear, acid in reaction, and its specific gravity ranges between 1012 and 1020; it often has a strong urinous odour, to which attention is sometimes directed by the patient himself. The absorption of food has been found to be very defective, as shown by the appearance of abnormal amounts of nitrogen in the fæces. Stejskal and Erben⁵ found that 17 per cent. of the nitrogen introduced in the food, 13.5 per cent. of the fat, 6 per cent. of the not easily absorbable carbohydrates, and 30 per cent. of the chlorides were evacuated in the fæces; still, at the end of four days, a slight positive N-balance (1.15 grms.) remained. The urea nitrogen represented about 85 per cent. of the total urinary nitrogen; the uric acid excretion was relatively rather high, 0.44 to 0.76 gm. In advanced cases the amount of urea becomes less, partly from imperfect absorption of food, and partly from derangement of metabolism, which is delayed or interrupted, so that intermediate products appear in the urine, such as leucin, tyrosin, lactic and diacetic acids, and acetone. Another product is ammonia, of which considerable excess is sometimes present in the urine; at others, it is below the normal. The defective assimilative power leads to excess of putrefactive processes in the intestines, so that large amounts of indoxyl compounds are formed which, along with other ether sulphates, are excreted in the urine; in one case, Hunter⁶ found the proportion of preformed sulphates to ether sulphates to be 3:1, in place of the normal 10:1. Diamines have also been found in the urine.

The effects of the hæmolysis are directly represented in the urine chiefly by the presence of two substances—urobilin and iron. The hæmolysis furnishes an abnormal amount of blood pigment, which the liver converts into bilirubin; this leads to the presence of an excess of urobilin in the intestines, and subsequently in the urine which

¹ Virchow's *Arch.*, 1898.

³ *Ibid.* 1900.

⁵ *Zeitschr. f. klin. Med.*, 1900.

² *Journ. of Physiol.*, 1898.

⁴ *Riforma Med.*, 1902.

⁶ *Pernicious Anæmia*, 1901.

in a great measure owes its dark colour to the large amount of urobilin it contains. The increased amount of iron in the urine is less constant. On one occasion, in each of three separate cases, Hunter found the amount of iron in the twenty-four hours to be 32.62 mgrms., 6.52 mgrms., and 1.00 mgrm. respectively. Hopkins¹ found 8.5 mgrms. on one occasion, and but a mere trace a few days after; he found only a trace in another case.

In **chlorosis** the alterations in the urine are but slight. The urine is usually light in colour and low in specific gravity, the quantity being above the average. The amount of urea is unaltered, or is very slightly increased; that of uric acid also remains constant, or it may be slightly diminished. The ether sulphates have been found to be increased, and in some instances excess of indican has been observed. In chronic chlorosis Cavazza² found no increase in urobilin, but it occasionally occurred in acute cases; he considers that the resistance of the blood-corpuscles is diminished, and that consequently physical exertion, cold and hot baths, and febrile conditions cause hæmolysis and increase of urobilin in the urine. In chlorosis Hunter found a diminished amount of iron, 1.76 mgrms., which is considerably below what he obtained in healthy urine (5.65 mgrms.); a like result was obtained by Bartoletti.

MALIGNANT DISEASE.

The nitrogen excretion in malignant disease varies with the amount of food that is eaten; the urea-N tends to be lowered in proportion to the total-N, the difference being made up by ammonia and extractives, which are both relatively and absolutely increased, especially the latter; in some instances there is no increase in the ammonia-N. Setti³ found that the extractive-N may exceed 10 per cent. of the total-N, and that the disparity increases as the disease advances. The excretion of uric acid often remains unaltered; but sometimes a considerable increase has been observed. Brandenburg⁴ found a large increase in the xanthin bases. Blumenthal⁵ considers that any increase is due to inanition, and that when inanition does not occur there is no increase in xanthin bases.

The most constant change in the urine of malignant disease is due to retention of the chlorides; this is usually not very distinct until the disease is well advanced, when it is both marked and constant.

¹ *Guy's Hosp. Reps.*, 1895.

² *Il Policlinico*, 1900.

³ *Rivista Veneta d. Sc. Med.*, 1899.

⁴ *Berliner klin. Wochenschr.*, 1896.

⁵ *Charité-Annalen*, 1896.

The excretion of phosphoric and sulphuric acids corresponds to the amount of food that is eaten and of the nitrogen that is excreted.

In some cases of malignant disease, especially when it affects the stomach and bowels, an increase in urinary urobilin has been observed, due to rapid hæmolysis. If it occurs it is generally in an advanced stage of the disease, but its occurrence is by no means constant. Braunstein¹ states that, in cases of carcinoma, excessive excretion of urobilin only occurs when the liver is invaded, and then only so long as the flow of bile is not impeded. In cancer of other organs, significant urobilinuria is only met with when complications arise, such as fever and secondary hepatic disease. Should the question of a diagnosis between malignant disease and pernicious anæmia arise, the early appearance of urobilinuria would point to pernicious anæmia, and the absence of it later on to malignant disease. Abdominal malignant disease is one of those conditions in which enormous amounts of indoxyl and, in a less degree, other aromatic compounds appear in the urine; a considerable excess also often occurs when the disease attacks other parts of the body. Blumenthal found skatol carbonic acid in several cases of carcinoma of the stomach and intestines. In the later stages of malignant disease acetone and diacetic acid are frequently present in the urine; β -oxybutyric acid has also been found in the final stage of coma (Klemperer²). When the disease is advanced, a small amount of albumin is more frequently present than absent. On many occasions albumoses are stated to have been present, but the evidence given is often far from convincing. v. Noorden limits the occurrence of albumosuria to cancerous growths which are ulcerating, the albumose being derived from absorption of the decomposing proteids present in the discharge. This coincides with my own experience. I have never detected albumose in the urine in cases of malignant disease, except when a large ulcerating surface was exposed and absorption of the discharge had taken place.

DISEASES OF THE DIGESTIVE ORGANS.

To some extent the activity of the peptic digestion is reflected in the urine: if the gastric glands secrete active pepsin abundantly, the urine possesses a certain degree of proteolytic power; any marked diminution of this, indicates faulty secretion on the part of the stomach. The acidity of the urine is diminished by many diseases of the stomach: in severe atonic dilatation which is usually accom-

¹ *Zeitschr. f. Krebsforschung*, 1903.

² *Congress f. inn. Med.*, 1889.

panied by profuse recurrent vomiting, the reaction of the urine approaches or reaches alkalinity, on account of the hydrochloric acid which is ejected being withdrawn from the system; in extensive malignant disease of the stomach, apart from vomiting, the acidity of the urine is reduced on account of retention of the chlorides. In these urines a deposit of phosphates frequently occurs, and if magnesia is administered, the normal magnesium phosphate usually appears. In acid dyspepsia and in chronic gastric ulcer the acidity of the urine is increased; an increase in acidity also occurs in exclusive rectal feeding, and after the administration of purgatives. (Moreigne.¹) In emaciated patients, when the diagnosis lies between atonic dilatation of the stomach and cancer, a urine poor both in chlorides and urea points to simple inanition; if whilst being poor in chlorides it is not abnormally low in urea, malignant disease is indicated.

Catarrhal and inflammatory affections of the stomach, and more especially of the intestines, conduce to putrefaction of their contents and consequently to the presence in the urine of excess of ether sulphates in proportion to preformed sulphates. From the same cause, large amounts of indoxyl-combinations, of urorosein, and possibly of skatoxyl-combinations, appear in the urine. It is to be observed that all the products of intestinal decomposition do not appear in the urine as ether-sulphates; amongst the exceptions are urorosein, the indol combinations of glycuronic acid, and those of skatol with carbonic acid. Stoppage of the bowels, ileus, tuberculous and malignant diseases of the bowels, and peritonitis, are conditions which cause the greatest excess of indol-combinations to appear in the urine; intestinal catarrh accompanied by profuse, mucous diarrhoea is another cause. Hæmorrhage into the small intestine produces excess both of indoxyl and of urobilin in the urine. In many of the conditions in which intestinal putrefaction occurs, its intensity may be lessened by the administration of certain drugs, notably by calomel, with the obvious result that the urinary chromogens of putrefactive origin are greatly diminished; ordinary purgatives do not act in this way. Nutrition accomplished by sterilised food produces little effect on the rate of intestinal putrefaction unless the bowel has been previously partially sterilised by calomel. Some conditions of the intestines which are due to, or are accompanied by, bacteria, may be causative of bacteriuria; in enterica, the typhoid bacillus may be present either alone or along with *Bacterium coli*; in many slightly abnormal intestinal conditions, the *Bacterium coli* appears in the urine.

Abnormal conditions affecting the liver may lead to various changes in the urine. In seven cases of gallstone colic, Gilbert and

¹ *Compt. rend. Soc. Biol.*, 1900.

Castaigne¹ found considerable diminution in the urea, in one instance it did not exceed 7 grms. in the twenty-four hours. In four of these cases urobilin was present. Alimentary glycosuria lasting for five or six days was observed in the four cases which were tested; in two of these indican was present, and persisted after the glycosuria had ceased. Gilbert and Castaigne attribute the occurrence of alimentary glycosuria in gallstone colic to inhibitory arrest of the functions of the liver. Bergenthal² obtained alimentary glycosuria in six out of twenty cases of gallstone colic. It has been stated that spontaneous glycosuria frequently accompanies gallstone colic. Exner³ declares that sugar was present in the urine of thirty-nine out of forty cases; this is obviously an error. Zinn⁴ obtained a positive reaction for glucose in only two out of eighty-nine cases; Kausch⁵ in only one out of seventy cases; and in 250 cases Naunyn⁶ never found glucose present in the urine, except in one, in which the liver was diseased. Alimentary glycosuria has been observed in various diseases of the liver, but it is of no practical import. In many of the recorded cases, there is reason to believe that the low assimilation-limit for sugar was due rather to the abuse of alcohol than to the disease of the liver: excessive drinkers readily succumb to alimentary glycosuria, and with them liver diseases are frequent. H. Strauss⁷ administered to each of twenty-nine patients with liver disease, and to fifty-eight persons who had no liver disease, 100 grms. of levulose on an empty stomach: in 90 per cent. of the former, and in only 10 per cent. of the latter, alimentary levulosuria occurred. Landsberg⁸ denies any causal relation between hepatic disease and alimentary levulosuria, which often occurs in perfectly healthy people; in twelve cases of hepatic disease he frequently obtained negative results. In jaundice, some of the volatile fatty acids are frequently present in the urine, and glycerinphosphoric acid has been found in cases of fatty liver when the bile-ducts are completely blocked.

Functional derangement of the liver may be the cause of increase in indoxyl and similar products. Gilbert and Weil⁹ state that indican is kept back by the healthy liver, and that when the function of that organ is deranged an abnormal amount of indoxyl may appear in the urine without the occurrence of undue intestinal putrefaction. Ajello¹⁰ associates the pancreas with the liver in this

¹ *Compt. rend. Soc. Biologie*, 1900.

³ *Deutsche med. Wochenschr.*, 1898.

⁵ *Deutsche med. Wochenschr.*, 1898.

⁷ *Deutsche med. Wochenschr.*, 1901.

⁹ *Compt. rend. Soc. Biol.*, 1899.

¹⁰ *Giorn. internaz. d. sc. Med.*, 1901.

² *Dissert.* Giessen, 1901.

⁴ *Centralbl. f. inn. Med.*, 1898.

⁶ *Der Diabetes mellitus*, 1898.

⁸ *Ibid.* 1903.

function. Rabaioli¹ believes that indicanuria may be indicative of liver insufficiency, although not specifically so, since it may be due to diseases of other organs. In cirrhosis the urea may be diminished and the ammonia increased; sometimes the output of uric acid is increased, and not infrequently there is excess of urobilin. In severe, destructive lesions of the liver, like acute atrophy, the excretion of urea is very much diminished, and intermediate products, such as ammonia, leucin, tyrosin, and some of the volatile fatty acids appear; the uric acid is increased, and also the xanthin bases. (Röhmman.²) Oxymandelic acid has been found. In a case of acute atrophy of the liver which was followed by recovery, Senator³ found the excretion of urea to be very much diminished; at one period the total nitrogen excretion was very high, indicating excessive proteid metabolism, whilst the relative urea-excretion was extremely low (69 per cent.). The ammonia-nitrogen was increased to three or four times the normal, more particularly at the period of the scanty urea-excretion. No special changes occurred in the excretion of the alloxur bodies. At the most critical period of the disease, acetone and diacetic acid were present in the urine. From this and other similar observations, it appears in certain diseases which are accompanied by extremely rapid proteid metabolism, and in which the liver is implicated, that scanty urea-excretion goes hand in hand with copious nitrogen-excretion.

In the second stage of acute phosphorus poisoning, the urea is diminished and the ammonia is greatly increased, owing to a large amount being required, in addition to the fixed bases, to neutralise the excess of acid which results from imperfect tissue metabolism. Leucin and tyrosin are much less frequently present in acute phosphorus poisoning than in acute atrophy. Sarcolactic acid and sugar have occasionally been found.

It is surprising how little change may be produced in the essential constituents of the urine by profound organic changes in the liver. In a severe case of cirrhosis, Richter⁴ found that the ammonia-nitrogen ranged between 3.6 and 9.7 per cent.; and the urea-nitrogen between 72.2 and 89.5 per cent., percentages which are but little removed from the normal. In a case of widely infiltrating carcinoma of the liver in which the disease involved almost all the abdominal organs, the resulting changes in proteid metabolism scarcely affected the composition of the urine: the urea was slightly diminished; the ammonia-nitrogen was increased, whilst the extractive-nitrogen was little if at all changed.

¹ *Il Policlinico*, 1900.

³ *Charité-Annalen*, 1898.

² *Berliner klin. Wochenschr.*, 1888.

⁴ *Charité-Annalen*, 1898.

In almost all disorders of the liver, excess of hæmatoporphyrin occurs in the urine; the amount that is frequently present in hepatic disorders, with or without enlargement, which are due to alcohol, and to cardiac disease, is quite remarkable. The same conditions lead to the presence of uroerythrin in excess, as does also cirrhosis; in cases of this description, the urates that are deposited have a bright orange-red colour, altogether different to their usual subdued pink appearance. An increased amount of urobilin usually attends hepatic derangements, and occasionally glycosuria occurs. Those disorders of the liver that arrest, or obstruct the passage of bile into the duodenum, give rise to the presence of bile-pigments in the urine, a condition that is elsewhere described.

SPECIFIC INTRINSIC INTOXICATIONS.

In addition to the ordinary forms of intrinsic intoxication, such as uræmia and diabetic coma, cases occur, apart from antecedent pathological conditions, in which a toxic agent is developed within the organism, and gives rise to a certain train of symptoms. These cases may be divided into two classes: (*a*) Cases in which the urine contains no sugar, but in which acetone is present in the breath, and acetone, diacetic acid, and, possibly, β -oxybutyric acid are present in the urine; (*b*) Cases in which none of these products are developed, but in which the urine contains an excessive amount of urinary indican. In both classes the toxic agent is probably formed in the intestinal canal; but the precise synthetic path taken by those portions of the intestinal contents which furnish the toxine is at present a matter of conjecture. Equally uncertain is it whether the intestinal metabolism is solely at fault, or whether the tissue-metabolism is also deranged; in many of these cases (but by no means in all) the activity of the general metabolism is reduced, especially as regards full oxidation, either on account of a low percentage of hæmoglobin (anæmia), or as the result of some less specific impairment.

In some of the cases in class (*a*) the symptoms coincided with those of diabetic coma; in others, tonic and clonic spasms constituted the leading clinical feature. In several cases seen by Dreschfeld (of which all but one were women), several being of neurotic temperament), persistent vomiting, headache, and extreme debility occurred, followed by coma with "air-hunger" like that of diabetic coma; the odour of acetone was present in the breath, and diacetic acid and acetone were present in the urine, but no sugar. One case died,

and death was preceded by oliguria and by a temperature of 103° F. In the urine from this case, and also in that from two other cases that recovered, Craven Moore¹ found acetone, diacetic acid, and β -oxybutyric acid, but no glucose. Filtered specimens of the urines in a two-décimètre tube rotated the plane of polarised light— 3° , 0.46 , 0.2 , respectively.

Edsall² saw a man, aged sixty-three, who was attacked whilst at work; he became unconscious and cyanosed, with deep, full respirations, without stertor, eighteen to twenty in the minute. The breath gave off a powerful odour of acetone, which, along with diacetic acid, was present in the urine. The patient remained unconscious for twelve hours and then recovered. v. Jaksch³ records the case of a man aged twenty-four, who was suddenly attacked with pain in the head and with colic, followed by tonic and clonic spasms; acetone and diacetic acid were present in the urine. Kraus⁴ relates the case of a woman who, after visceral disturbances, became dull and stupid and died comatose; on the day of her death the urine contained 2.5 per cent. of β -oxybutyric acid. Post-mortem examination revealed no intracranial lesion, but there were indications of gastro-intestinal catarrh. Lorenz⁵ observed acetonuria in various gastric disorders: catarrh, ulcer, dilatation, gastric crises, and gastro-enteritis; also in hysteria and chronic plumbism. In some cases he observed meningeal-like symptoms. Edsall⁶ records cases of recurrent vomiting in children, probably due to acidosis, which was cured by the administration of twenty-grain doses of sodium bicarbonate. Morfan⁷ doubts the acidosis theory, and prefers to call such cases "vomiting with acetonæmia." Mohr,⁸ following v. Noorden, lays emphasis on the importance of the food-carbohydrates in the prevention of acidosis, and attributes many of these cases to a condition comparable with "hunger diabetes," in which intermediate products, such as glycuronic, or lactic acids, are formed in consequence of deficient supply, or of faulty metabolism of the carbohydrate food-stuffs.

Class (b) is characterised by absence of acetone, and by the presence of large amounts of urinary indican. It is doubtful if the indican is the sole representative of the toxic agency; there are reasons for believing that tox-albumins may be formed by micro-organismal action, alongside the indol, and that they are responsible for the symptoms, or, at least, for many of them. Several of the cases

¹ *The Lancet*, 1903.

² *Philadelphia Med. Journ.*, 1902.

³ *Zeitschr. f. klin. Med.*, 1886.

⁴ Lubarch and Ostertag's *Path. Anat. u. Physiol.*, 1895.

⁵ *Zeitschr. f. klin. Med.*, 1901.

⁶ *American Journ. Med. Sc.*, 1903.

⁷ *Arch. de Méd. des. Enfants*, 1901.

⁸ v. Noorden's *Samml. klin. Abhandlungen*, 1904.

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observed by the author occurred in children, the symptoms bearing a strong resemblance to those of meningitis: retraction of the head, contracted pupils, occasional clonic spasms, vomiting, stupor, and collapse, followed by rapid recovery. In a woman the symptoms were extreme prostration, persistent vomiting, dilated insensitive pupils, heavy stupor, but not actual coma, which continued for more than thirty-six hours, when very gradual recovery took place with a partial remission. In all these cases there was obstinate constipation, the motions eventually procured being extremely offensive; the urine contained enormous amounts of urinary indican, which in some instances came down in flakes of indigo on the addition of oxidising agents. After a few doses of calomel the motions became natural, and the urine yielded a mere trace of indigo. Stuertz¹ records the case of a youth of seventeen who was attacked with abdominal pain, vomiting, constipation, slow pulse with high tension, unconsciousness, and clonic spasms with trismus. The pupils were widely dilated and insensitive to light. The knee-jerk was exaggerated; the temperature was 100.4° F. Great excess of indican was present in the urine. Recovery took place after the bowels were evacuated with the aid of calomel; the stools were very offensive.

DISEASES OF THE KIDNEYS.

Acute nephritis.—The quantity of the urine is diminished; it does not usually exceed twelve or eighteen ounces, and it may be limited to an ounce or two, voided a few drops at a time with much straining; sometimes there is complete suppression. The colour is either that of blood mixed with water, or it is a dirty brown; in mild cases and in the defervescent stage of severer attacks it may be "smoky." There is a heavy dark-brown, or reddish deposit which consists of casts, blood corpuscles, leucocytes, epithelial cells, granular matter and probably urates; occasionally, the urates are partially or wholly kept in suspension by the presence of an excess of globulin in the urine. The casts may be hyaline, epithelial, or granular; in hæmorrhagic nephritis blood-casts are common. The reaction of the urine is usually acid; its specific gravity is higher than that of healthy urine, ranging from 1025 to 1040. The amount of albumin varies from less than one per cent. up to an amount that is sufficient to cause the urine to solidify when boiled. The urea is diminished; it may be 50 per cent. below normal. Uric acid is diminished in the early stage; subsequently it is

¹ *Berliner klin. Wochenschr.*, 1903.

increased. (Kam.¹) The chlorides, and, in a less degree, the sulphates and the phosphates are diminished.

Chronic nephritis.—The quantity of urine is usually below normal, and its colour is rather high. After the subsidence of an acute attack, which has merged into the chronic form, the urine may be dusky, or smoky at times, or it may become pale, almost colourless, with a faint tinge of red, best seen in the deposit, which in such cases is light-coloured with a reddish tint in the upper stratum. In the passive chronic stage, the colour of the urine may differ little from that of the healthy secretion. The deposit is much heavier than that of normal urine; it consists of casts, epithelial cells, leucocytes, stray red corpuscles and much granular matter; later on, it may become lighter in colour and may contain a large quantity of leucocytes and fatty cells. At an advanced stage, fat-crystals may occasionally be seen projecting from fatty casts. The specific gravity is rather higher than in healthy urine, from 1018 to 1030. The amount of albumin varies from 0.05 to 0.3 or more per cent. The percentage of urea runs parallel with the severity and the stage of the disease; in the mid-period it may be little below normal, but as the changes in the kidneys advance it may be reduced to below one per cent. The excretion of uric acid differs little from the normal; Kam found it a shade higher. v. Jaksch² states that pronounced retention of urea may occur without compensatory increase in the purin and amido acid-nitrogen; in some instances, however, there may be such an increase. Halpern³ found that the extractive-nitrogen is increased, but that there is no alteration in the amido acid-nitrogen. Mohr⁴ thus epitomises the results of investigations on the excretory power of the diseased kidney which, apart from clinical and anatomical differences, are in general agreement on corresponding points: varying excretion of water, nitrogen and salts; good excretion of ammonia and the purin bases. The chlorides undergo a reduction which keeps equal pace with that of the urea; in an advanced stage of nephritis when uræmia threatens, the diminution of the chlorides becomes very marked. Marischler⁵ found in cases of parenchymatous nephritis, even with diminished diuresis, that the kidneys were readily permeable to sodium chloride; he explains the eventual diminution in the excretion of chlorides to the retention of water. Strauss,⁶ on the contrary, regards retention of sodium chloride as a trustworthy sign of kidney insufficiency, and further, that it

¹ *Diss. Leiden*, 1898.

² *Loc. cit.*

³ *Zeitschr. f. klin. Med.*, 1903.

⁴ *Ibid.*

⁵ *Arch. f. Verdauungskrankh.*, 1901.

⁶ *Therapie der Gegenwart*, 1903

is the cause of the formation of œdema. Mohr¹ believes that the sodium chloride retention and the water excretion have no interdependence. Charrier² found gradual and progressive retention of potassium, amounting in some cases to two-thirds; in those patients who vomited, potassium was present in the vomited matter. On the other hand, in cases about to terminate fatally, Herringham³ found almost complete retention of sodium without corresponding diminution of potassium. The excretion of phosphoric acid is liable to undergo considerable oscillations in daily amount; the excretion of sulphur also varies, but in a much less degree.

In an advanced stage of Bright's disease the kidneys become impervious to some of the urinary pigments, and when this occurs urochrome, urobilin, and uroerythrin are absent, or nearly so, from the urine; albuminuria and urobilinuria rarely occur together. In advanced chronic nephritis, the urea may be below one per cent, and yet for a time an approximate balance of urea-excretion may be maintained by copious excretion of urine. In such cases the specific gravity of the urine is low—1006 to 1008—and it is almost colourless. Klemperer⁴ points out that scanty, and at the same time light-coloured urine, is indicative of severe kidney disease; the scanty urine of heart disease is high-coloured as long as the kidneys are fairly competent; when the urine is both scanty and light-coloured the prognosis is bad.

In the section on kryoscopy it is stated that the molecular concentration of normal urine ranges over such wide limits as to preclude the determination of a standard freezing-point; the kryoscopic method, however, may be made to yield information as to the functional activity of the diseased kidney. With healthy kidneys, when a large quantity of water is swallowed, a rapid elevation in the freezing-point of the urine occurs, possibly amounting to a degree and a half centigrade; in many cases of Bright's disease, under like conditions, the freezing-point remains constant, or is but little altered. In some instances, the diseased kidneys acquire a compensatory power and, under the stimulus of an excess of ingested liquid, act much in the same way as the normal gland; in them the polyuria is not attended by a parallel excretion of molecules of osmotic activity. Roeder⁵ points out the necessity of conducting kryoscopic investigations on the urine in renal disease, in association with predetermined dietaries, especially as regards the amount of

¹ *Loc. cit.*

³ *Brit. Med. Journ.*, 1903.

⁵ *Ibid.*

² *Compt. rend. Soc. Biol.*, 1897.

⁴ *Berliner klin. Wochenschr.*, 1903.

liquid they comprise. By allowing the patient to drink forty to fifty ounces of water on an empty stomach and then determining the freezing-point of successive portions of the urine during the ensuing five hours, the osmotic action of the kidneys may be ascertained. In this way, Kövesi and Roth-Schulz¹ found that, in the healthy kidney Δ rose to -0.1° C.; in renal congestion due to uncompensated heart disease the elevation was less, and in subacute parenchymatous nephritis it was either very much less, or was entirely absent, according to the severity of the disease. In primary contracting kidney, and in compensated heart disease Δ was little if at all affected. Senator² does not find the distinction between the two types of nephritis so well marked.

Granular kidney.—The quantity of urine is above normal, and varies from 50 or 60 ounces to 200 or more in the twenty-four hours. The casual statement of a patient that he has to get out of bed every night to empty his bladder, should always rouse suspicion; for, in the absence of diabetes and of irritable bladder the habit is very suggestive of granular kidney. The urine is light-coloured, and there is very little deposit on standing. The reaction is acid. The specific gravity is low, 1005 to 1014. The amount of albumin is small, often merely a trace, and occasionally it may be absent; therefore the urine from a case of suspected granular kidney should always be examined by the boiling-test, and, in the event of a negative reaction, other specimens should be examined before a definite diagnosis is made. The percentage of urea is below normal, but this is compensated by the increased volume of urine, so that the daily out-put is not diminished; irregular variations in the daily excretion of nitrogen are common in interstitial nephritis: sometimes it may be below, and at others in excess of the normal average. Troitzki³ found a relative diminution of urea, with normal excretion of uric acid and creatinin; the balance of the nitrogen is represented by ammonia and the xanthin-bases, which are relatively increased. Casts are rare; occasionally one or two hyaline casts, or possibly a granular cast may be observed. Sometimes crystals of uric acid, or of calcium oxalate are present. The chlorides are diminished. The phosphoric acid excretion is subject to considerable variations; usually it is diminished; the sulphates have also been found to be diminished. In six fatal cases of granular kidney, Herringham⁴ found sodium either present in very small amount, or altogether absent; in five other cases which did not die, no alteration occurred. West⁵ directs attention to the occasional occurrence of hæmaturia

¹ *Orvosi Hetilap*, 1900.

² *Deutsche med. Wochenschr.*, 1900.

³ *Botkin's Krankenhausztg.*, 1900.

⁴ *Loc. cit.*

⁵ *On Granular Kidney*, 1900.

in granular kidney, sometimes so copious as to suggest the bladder as its source; but although the urine may look almost like pure blood, no clots are present. A milder form of hæmaturia is less infrequent. In the later stage of granular kidney, the quantity of urine is diminished, its colour becomes deeper, its specific gravity higher, and it contains more albumin and casts.

Amyloid kidney.—The urine resembles that secreted by the granular kidney. It is copious, 100 to 200 ounces in the twenty-four hours; it is pale in colour, and has a specific gravity of 1010 or lower. In the early stage the amount of albumin is small, often merely a trace; later on it increases, and the volume of the urine decreases. Epithelial, fatty, and possibly waxy casts may be present. The urea and the chlorides are diminished; the phosphoric and sulphuric acids are reduced, but in a less degree.

Uræmia.—The urine contains albumin, is scanty, and, usually, of low specific gravity. Nitrogen-retention commonly occurs. The output of urea has been observed to be diminished before an attack of uræmia, and increased during and after the attack. Richter¹ found the extractive-nitrogen to be increased to 24 per cent., or more than double, shortly before and during the uræmia; the ammonia-nitrogen gradually increased up to the day when the patient became comatose, when it suddenly went up to 17 per cent. and then sank to from 10 to 7 per cent. In one case the daily intake of nitrogen was exceeded by the output, which is in favour of v. Noorden's view that, in acutely occurring uræmia toxins, which have a deleterious action on cell-life, circulate in the blood. Bouchard² held that toxic products, which are normally excreted in urine, are retained in uræmia, and that the urine is consequently less toxic than it is in the normal state; but, as previously explained (page 200), the toxicity of urine is probably not due to the presence of toxins. Stern³ suggests a physico-electric substratum in the pathogenesis of the uræmic state. He states that none of the substances which are retained in uræmic serum possess intrinsically poisonous properties, and directs attention to the extraordinarily high osmotic pressure of uræmic serum which is not due to its ions but to the presence of neutral molecules. Molecular conductivity is independent of molecular concentration, so that although uræmic serum has a high molecular concentration (as determined by its freezing-point), its conductivity is considerably below that of normal serum, because the neutral molecules or non-electrolytes hinder complete dissociation and diminish the movements of the ions. By

¹ *Charité-Annalen*, 1898.

² *Compt. rendus*, 1886.

³ *New York Med. Record*, 1903.

intravenous injections of water, the blood serum is diluted and its conductivity is consequently improved; this is more easily accomplished in cases of parenchymatous, than of interstitial nephritis, as there is less nitrogen-retention in the former than in the latter. Strauss¹ states that the average nitrogen-retention (not proteid-N) in the blood serum of chronic parenchymatous nephritis equals 62.3 mgrms. in 100 c.c.; whilst in interstitial nephritis it reaches 129.7 mgrms. He finds that in neither of these types of nephritis does the freezing-point materially differ from the normal. Engelmann² finds that the electrical conductivity of the blood remains unchanged in uræmia. Diminished alkalinity of the blood in uræmia was observed by v. Jaksch³ who is disposed to regard acidosis as a possible cause. Orłowski⁴ also found greatly diminished alkalinity of the blood, to 42 and 46 per cent. in uræmic patients, not, however, at the commencement of the attacks, nor when they had reached their fullest intensity, but during their subsequent course; from this he infers that the accumulation of acids in the blood is not the cause, but rather the result of uræmia, as expressing the profound disturbance it produces on metabolism.

The duration of the albuminuria of kidney disease.—It has been previously stated that albuminuria may exist for considerable periods without the presence of renal disease; it is equally true that the albuminuria due to organic disease of the kidneys may also run a very prolonged course. It is by no means uncommon for patients with chronic nephritis, in whose urine albumin is constantly present, to live and enjoy good health for ten or more years. It is exceptional for the albuminuria to cease after having persisted for years, but Johnson⁵ recorded the case of a man aged twenty-six whose urine after an attack of scarlet fever was continuously albuminous for more than six years; by strict diet the urine became free from albumin, and remained so eighteen months later. The author⁶ reported an ultimately fatal case of prolonged albuminuria in which a girl aged fourteen was attacked with scarlet fever, followed by severe parenchymatous nephritis, and, during the remaining twenty-eight years of her life, she was subject to continuous albuminuria, the urine was examined six or eight times every year, and was never found to be free from albumin, which varied from 0.2 to 1 per cent. Until the last year of her life she retained her rosy complexion,

¹ *Die chronische Nierenentzündungen*, 1902.

² *Münchener med. Wochenschr.*, 1903.

⁴ *Przegląd lekarski*, 1901.

⁶ *The Lancet*, 1895.

³ *Zeitschr. f. klin. Med.*, 1888.

⁵ *Brit. Med. Journ.*, 1879.

and never became anæmic; she was moderately stout, and remained so. She died from uræmia. The kidneys were examined by Delépine, who found that both presented all the appearances associated with the term granular kidney. Wilkinson¹ records the case of a man aged fifty who, thirty years previously, had scarlet fever; since then, whenever his urine was examined it was found to contain albumin, and still his general health remained good.

DISEASES OF THE NERVOUS SYSTEM.

In those diseases of the nervous system to which the term "functional" is applied, quantitative changes in the urine are often observed. Attacks of hysterical excitement are followed by copious polyuria of short duration, and in all patients of the neurotic type any nerve-tension may induce a milder degree of polyuria which may continue for days or for weeks. In such cases the urine is light in colour and of low specific gravity, differing from the normal excretion only by its extreme dilution. Less frequently, on the other hand oliguria may occur; even complete anuria has been met with in hysterical women; a condition not to be confounded with the frequently encountered hysterical retention of urine. In that ambiguous condition to which the term neurasthenia is applied, the urine often manifests certain peculiarities: it is copious, light in colour, of low specific gravity, of alkaline or amphoteric reaction, and either spontaneously, or after being heated, it deposits phosphates. In some cases of neurasthenia a different condition of the urine is met with; de Fleury² observed diminution in volume, with highish specific gravity, and excess of uric acid in relation to urea, and of earthy phosphates to the alkali phosphates; he also noticed an increase in the chlorides, and lowering of the oxidation coefficient. In severe cases Bechterew³ also found more or less diminution of urea, and in most cases, great excess of uric acid; the relation borne by the total nitrogen of the urine to the urea-nitrogen indicated a marked depression in the activity of the nitrogenous oxidation. The relation borne by the total nitrogen to the phosphoric acid was frequently deranged in such a way as, according to Zuelzer's theory, to indicate excessive degeneration of tissues rich in phosphorus, represented in these cases by the brain. This explanation of the occurrence of an excess of phosphates in the urine in cases of nerve-disease was first given by Bruce Jones in relation to meningitis; it is doubtful, however, how far it is applicable to the condition under discussion. As

¹ *The Lancet*, 1895.

² *Bull. gén. de Thérap.*, 1900.

³ *Neurolog. Centralbl.*, 1899.

the result of a series of interesting investigations on this subject, Iwanoff¹ found that in so-called phosphaturia the amount of phosphoric acid is below rather than above the normal; this agrees with the results obtained by Panek (see page 21). Iwanoff also found that proteid food, and foods that are rich in lime, increase the turbidity of the urine, whilst vegetable food reduces it, partly on account of the small quantity of lime and magnesia salts it contains, and also because it favours the excretion of these earthy metals by the bowel. The administration of magnesium salts determines an increase in the amount of calcium excreted by the bowel, and consequently diminishes the absorption and subsequent elimination of calcium in the urine; at the same time, the amount of magnesium in the urine is increased. The turbidity of the urine in these cases of misnamed phosphaturia, therefore, is due to excessive excretion of calcium salts. Freudenberg² finds that ammonia is always present in urine that is turbid from excess of earthy phosphates, and that apart from "phosphaturia," ammonia may be present even though the reaction of the urine is acid; on boiling such urine the vapour given off contains ammonia. He regards this as an indication of some significance in the diagnosis of neurasthenia. Very exceptionally, in cases of neurasthenia in which the urine is alkaline, small spherical and hour-glass crystals of calcium carbonate may be seen under the microscope.

After attacks of epilepsy, traces of albumin and, very exceptionally, of glucose may be present in the urine. An increase in the amount of urea, uric acid, and the chlorides has been observed. By keeping epileptics exclusively on milk, which contains rather less than a gramme of sodium chloride in the litre, Roux³ demonstrated the accuracy of Richet's and Toulouse's⁴ statement that in epilepsy the efficacy of potassium bromide is increased by diminishing the intake of chlorides. Schlöss⁵ also found that the number of the attacks is lessened by the simultaneous administration of bromides and of food that is poor in chlorides; but that the body-weight is reduced and the patient becomes weak; without the bromides, no effect is produced on the frequency of the attacks by milk diet. In several long standing and severe cases of epilepsy, Andenino and Bonelli⁶ found that after the attacks there was little if any increase in the earthy phosphates of the urine, but that a considerable excess of calcium was present in the faeces. On the other hand, in recent cases, they found that excess of earthy phosphates occurred in the urine, but not in the faeces, and

¹ *Russki Wratsch*, 1903.

² *Deutsche med. Wochenschr.*, 1903.

³ *Compt. rend. Soc. Biolog.*, 1900.

⁴ *Ibid.* 1899.

⁵ *Wiener klin. Wochenschr.*, 1901.

⁶ *Giornale d. R. Accad. di Med. di Torino*, 1902.

that simultaneously with the absorption of the calcium the motor attacks ceased. Inouye and Saiki¹ found a large amount of lactic acid in the urine after attacks of epilepsy, which they attribute to diminished supply of oxygen during the attacks, and not to deranged liver-function.

Albumin is frequently found in the urine after attacks of apoplexy, in cerebral growths and in various inflammatory processes affecting the brain. Occasionally sugar may also be present, most frequently when hæmorrhage takes place into the fourth ventricle. The glycosuria, usually, is only transient; but in cases of tumour of the pons, or medulla, or in the floor of the fourth ventricle, it may persist. Sugar is not unfrequently present in the urine in some chronic diseases of the nervous system, such as tabes, general paralysis, disseminated sclerosis, tumour of the spinal cord (also hæmorrhage), myelitis, and syphilitic diseases of the nervous system. In Graves' disease, polyuria frequently occurs with or without the presence of sugar; the excretion of phosphates is often in excess, and there may be an increase in the urinary nitrogen. In acromegaly (tumours of the pituitary body), glycosuria has been observed in about one-third the number of recorded cases; in two cases investigated by Williamson² the sugar only appeared at a later period of the disease.

In various forms of mental derangement—delirium tremens, paranoia, and melancholia, sugar has been found in the urine. In five out of twenty-one cases of melancholia, Arndt³ detected alimentary glycosuria. As the result of investigations on the sugar-assimilation limit in the insane, Raimann⁴ comes to the conclusion that in inherited psychoses the assimilation limit is lowered; whilst degenerative psychoses show a high assimilation limit.

MICRO-ORGANISMS.

Bacteriuria.—Freshly voided, normal urine does not contain any micro-organisms; subsequently they may rapidly develop, but this does not constitute bacteriuria. By bacteriuria is meant a condition, first described by W. Roberts,⁵ in 1881, in which the urine contains bacteria before it leaves the bladder. The appearance of urine charged with bacteria is peculiar and distinctive; the urine is slightly turbid, and when agitated in a urine-glass by stirring it with a rod a wavy sheen is produced, which quickly disappears as the movement set up in the urine ceases. The bacteria do not subside when

¹ *Zeitschr. f. physiol. Chem.*, 1903.

² *Diabetes Mellitus*, 1898.

³ *Deutsche Zeitschr. f. Nervenkrankh.*, 1898.

⁴ *Arch. f. Heilkunde*, 1902.

⁵ *Brit. Med. Journ.*, 1881.

the urine is allowed to stand, and consequently it usually remains turbid until decomposition sets in. No change is produced in the appearance of the urine by passing it through ordinary filter paper, as it does not retain the bacteria. Very exceptionally, the bacteria may be carried down by a small excess of mucus derived from slight vesical catarrh; they then form a thin white layer on the bottom of the containing-vessel, leaving the urine perfectly clear. When bacterial urine is tested with a sensitive reagent, a trace of proteid may generally be found; usually it is a compound proteid.

Two kinds of bacteriuria occur: (a) simple, idiopathic bacteriuria, in which no other abnormality is discoverable, the micro-organism usually being the *Bacterium coli commune*. This type of bacteriuria is very irregular in duration; it sometimes occurs for a day or two, and then spontaneously disappears, possibly recurring subsequently in a like fugitive manner. On the other hand, it may persist continuously for months; the author watched a case for three years, in which bacteria were never known to be absent from the urine. But for the appearance of the urine in these cases the condition would escape notice, as no vesical or urethral irritation is necessarily produced.

(b) Bacteriuria that is associated with some pathological condition. In many general diseases, especially if accompanied by profound enfeeblement, bacteriuria is common; even under these conditions it may come and go in a very erratic fashion; the micro-organism is usually of the *coli commune* group. *B. coli commune* is by no means always harmless; it is reported to have caused acute urethritis which simulated gonorrhœa. (Playm and Laag.¹)

The *Bacillus lactis aerogenes*, which occurs in the intestines occasionally finds its way into the urine and may be the cause of cystitis or of urethritis (Warburg²). As the result of catheterisation, with subsequent cystitis, Horton-Smith³ found in the urine *Bacillus proteus urinæ*, which differs from other members of the proteus group. During the acute stage of gonorrhœa, gonococci may be found in the urine, along with threads of muco-pus; in the chronic stage, they are found with difficulty, or they may be absent. Staphylococci have caused bacteriuria after an attack of gonorrhœa. (Sée.⁴)

The presence of *tubercle bacillus* in urine is indicative of tuberculous deposit, or ulceration in the kidney, or other parts of the genito-urinary tract; this bacillus has also been found in miliary tuberculosis without implication of the urinary organs. In cases of actinomycosis affecting the urinary tract, *Actinomyces* have been found in the urine,

¹ *Centralbl. f. Bakteriöl. u. Parasitenk.*, 1895.

² *Münchener med. Wochenschr.*, 1899. ³ *Journ. Path. and Bacteriol.*, 1897.

⁴ *Annales d. mal. d. org. gen.-urin.*, 1899.

but only very exceptionally. The *typhoid bacillus* is not infrequently present in the urine of patients who are suffering from enterica; it has been found in the early stage, but is more common after the first or second week; it may continue to be present for many weeks or even months.

Various pathogenic micro-organisms which have relation to the diseases from which the patients are suffering, have been found in the urine: in septic endocarditis, erysipelas, and other septic conditions, *Staphylococcus pyogenes aureus*, *Streptococcus pyogenes*, and occasionally other cocci are present. The spirillum of relapsing fever has been detected. In female children affected with thrush, the spores and threads of *Oidium albicans* may occur in the urine if the disease has attacked the vulva.

Among non-pathogenic fungi are *Sarcinæ* which, when abundant, form a flocculent, greyish-white deposit after the urine has stood for some time; sarcinæ occur in both acid and alkaline urines, and they are smaller and paler than the *Sarcinæ ventriculi*, probably on account of the medium in which they are grown. They are usually, if not always, accidentally introduced into the bladder by catheterisation. Finlayson¹ reports a case in which sarcinæ were present in the urine of a man for fifteen years without causing any bladder symptoms.

In dilatation of the stomach, Stein² observes that if sarcinæ are present in the contents of the stomach, the urine is usually acid; in dilatation with absence of sarcinæ, the urine is alkaline. This is not invariably the case; the author has found the urine to be alkaline, and to deposit earthy phosphates copiously, in dilatation accompanied by sarcinæ.

When a specimen of urine is required for bacteriological examination, the parts which surround the urinary orifice should be thoroughly cleansed and the urine then passed into a flask that has been previously sterilised by heat; after the urine has been voided, the flask is at once plugged with cotton wool. In the case of female patients, the urine should be transferred from the bladder to the flask by means of a thoroughly sterilised catheter, preferably constructed of glass. The urine is subsequently placed in a cylindrical vessel with a blunt, cone-shaped bottom, and allowed to deposit; if the suspended matter is scanty, and always if minute micro-organisms are being sought for, the centrifuge should be used. The deposit is then stained and examined, or cultivations from it are obtained, after the methods adopted in bacteriological research.

¹ *Brit. Med. Journ.* 1891.

² *Arch. f. klin. Med.*, 1876.

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