available sulphuric acid, the excess appears as compound glycuronate (see below). Phenol is not found in the urine of the new-born, because their intestine is sterile, nor is it found in the urine of animals whose intestine is maintained sterile from birth. All local putrefactive and gangrenous processes lead to the elimination of urinary phenol.

Phenol appears in quantities that vary from 0.017 to 0.5 gm. (Munk) in the urine. The average excretion is 0.03 gm. It is more abundant on a vegetable than on an animal diet. Phenol is increased in all conditions of coprostasis, particularly in the lower portions of the small intestine and in the colon; in peritonitis, in pyæmia, in suppurative processes, in phosphorous poisoning, and in diabetes mellitus. Tyrosin and benzol given by mouth also lead to increased phenoluria. When the urine contains much indican, it also always contains much phenol; but the reverse is not the case. As the methods employed for determining phenol also include kresol, the figures given really indicate the combined excretion of the two aromatic alcohols.

Detection: Phenol itself gives a large number of very sensitive tests. Unfortunately, however, more of these reactions give satisfactory results with phenol as conjugate sulphate, *i.e.*, with urinary phenol. In order to detect its presence the phenol must first be liberated as follows: One litre of urine is treated with so much H_2SO_4 that the strength of the mixture is five per cent. The solution is then distilled until the distillate gives no clouding with bromine water. This distillate contains phenol. The following tests can be performed: (*a*) Bromine water is added until the solution is yellow; on standing a crystalline, yellow precipitate of tribromphenol is deposited. (*b*) Millon's reaction is positive (Millon's reagent: Mercury is dissolved in an equal portion by weight of sixty-three per cent. $HNO_3(4HNO_3 + 3H_2O)$ first in the cold, then by warming. One volume of this solution is diluted with two volumes of water and the precipitate that forms filtered off. The clear filtrate is the reagent). With phenol this reagent gives a rose-red color. (*c*) Phenol treated with sodium hypochlorite gives a blue color; care must be taken not to add too much hypochlorite. (*d*) Heating phenol with iodine and sodium-hydrate solution to 50 to 60° C. gives a dark-red amorphous precipitate of triiodine phenol. The latter reaction has been employed in a quantitative method for determining phenols, the amount of mono-oxybenzols being calculated from the amount of iodine that is employed. The method is complicated and does not indicate individual phenols, but merely the phenols as a group. The reaction that occurs may be schematized by the equation:

$$\underset{\text{Phenol.}}{C_6H_5OH} + \underset{\text{Iodine.}}{6I} = \underset{\text{Triiodine phenol.}}{C_6H_2I_3OH} + \underset{\text{Hydriodic acid.}}{3HI}$$

Kresol.—Kresol, as has already been stated, is the most abundant of the phenols. Much that has been said of phenol, particularly in regard to occurrence and detection and estimation, applies with equal force to kresol. Kresol is a methylated phenol. The relation between the two may be illustrated by the formulas: $H.C_6H_5.OH$, phenol, $CH_3.C_6H_5.OH$, kresol.

There are three kresols that are isomeric, viz., parakresol, orthokresol, and metakresol, as the substituting methyl group may occupy any one of three different positions in the phenol ring. Of the three isomers parakresol is the most abundant in man; some orthokresol is usually also present. Metakresol has so far not been found in human urine, but it occurs in horses. The differentiation of kresols from phenol is possible, but the process is complicated. Clinically this differentiation is unimportant; hence it will not be described.

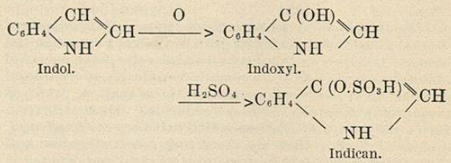
Pyrocatechin, $C_6H_4(OH)_2$.—This is a constant constituent of human urine. It is derived from pyrocatechu acid, that is of common occurrence in many plants. It is excreted after the administration of carbolic acid or of benzole.

Urine containing pyrocatechin soon turns dark on standing, provided the reaction is alkaline. The presence of pyrocatechin may be suspected if this change in the urine occurs, but hydroquinone and alkaptonic acid (see below) produce a similar change. A few drops of chloride of iron added to the urine give a dark green color if pyrocatechin is present. If a little tartaric acid is added and the mixture is treated with ammonia, the urine first turns violet and on addition of more ammonia cherry-red.

Hydroquinone, $C_6H_4(OH)_2$.—This body only occurs in the urine after the administration of carbolic acid (phenol), benzol and hydroquinone itself. It always appears as conjugate sulphate. Urine containing hydroquinone turns dark on standing. If urine containing hydroquinone is boiled with a dilute solution of iron chloride, quinon is formed, and this body can readily be recognized by its penetrating odor.

As pyrocatechin and hydroquinone frequently appear in the urine after carbolic-acid poisoning, it is important occasionally to isolate them from the urine in order positively to demonstrate their presence. For this purpose the urine is acidulated with hydrochloric acid, heated for half an hour on the water-bath, and after cooling extracted with ether. The ethereal extract is repeatedly shaken with sodium-carbonate solution, the ether evaporated and the residue treated with small quantities of a saturated sodium chloride solution. This salt solution takes up the pyrocatechin or hydroquinone that may be present. The solution is diluted with water and distilled to remove volatile phenols. The residue is again extracted with ether and the ether evaporated; the residue is dissolved in water and precipitated with lead acetate; the precipitate contains the pyrocatechin, while the hydroquinone remains in solution. The precipitate is decomposed with H_2SO_4 and extracted with ether. From this ethereal solution pyrocatechin crystallizes in prisms. The solution containing hydroquinone is treated with H_2SO_4, heated with barium carbonate, and the filtrate extracted with ether. From the ethereal extract hydroquinone crystallizes in the form of rhomboid crystals that can be recrystallized from benzol and melt at 160°.

Indoxyl (*Indican*), C_8H_7NO. Indican, the sulphate of indoxyl in combination with sodium or potassium, was originally believed to be identical with vegetable indican, the chromogen of the indigo plant. It has been shown, however, that animal indican is derived from indol and is the ethereal sulphate of indoxyl, an oxidation product of indol, while vegetable indican is a glucoside of the formula $C_{26}H_{31}NO_{17}$. The relation of indol, indoxyl, and indican may be illustrated by the following scheme:



Indol, like the phenols, is a product of the putrefaction of albumin somewhere within the organism. Consequently, we always find phenol in abundant quantities whenever indican is excreted in the urine. Inversely, however, the presence of phenol does not necessarily indicate the presence of indican. In the new-born indican is never found in the urine. In adults it is always present in small quantities. From 2 to 50 mgm. of indigo-blue can be isolated from the twenty-four hours' urine on a mixed diet. A meat diet leads to an increase of the urinary indican, and a vegetable diet to a decrease. Pathologically indicanuria is increased in all conditions that favor abnormal intestinal putrefaction, particularly if there is at the same time some stasis of the intestinal contents. Thus marked indicanuria is common in ileus, typhoid, acute and chronic peritonitis, intestinal tuberculosis. In cholera, gastric ulcer, gastrectasis, gastro-

intestinal catarrh, lead colic, perityphlitis and appendicitis indican has also been found increased in the urine. Occlusion of the colon does not lead to increased indicanuria. Large quantities of indican have finally been reported in cases of carcinoma of the liver, the stomach, the uterus, in empyema and putrid bronchitis, and in melanosis. Fever *per se* exercises no effect on the excretion of indican. Opium does not increase indicanuria. Laxatives, however, reduce it.

It will be seen, therefore, that indicanuria is a normal phenomenon, and that the excretion of large quantities of indican is a very common sign. Diagnostically, therefore, the appearance of abnormally large quantities of indican in the urine indicates merely that there is abnormal putrefaction of albumin somewhere in the body. Further than this we can rarely go, and it is a precarious matter to make a special diagnosis on the basis of increased indicanuria, for we must never forget that simple coprostasis may lead to a great increase of the urinary indican.

Other products of indoxyl that may appear in the urine are indigo red (indoxyl *plus* isatin), indoxyl glycuronic acid (see above), and a variety of more or less defined pigments that are variously known by the names urohæmatin, urrhodin, urorubin, indirubin, indigo-purpurin, etc.

Tests for Indican. 1. Jaffé's Test.—Hypochlorite of sodium oxidizes indican in acid solution to indigo blue. This pigment is soluble in chloroform. Equal parts of the urine and of concentrated hydrochloric acid are mixed in a test tube, and to the mixture are added a few cubic centimetres of chloroform and, drop by drop, a dilute solution of sodium hypochlorite. On the addition of each drop of the latter reagent the mixture should be thoroughly shaken. The layer of chloroform will gradually turn blue. Care must be taken not to add too much of the hypochlorite, as otherwise the indigo blue is oxidized further, and is converted into a colorless compound. Instead of using hypochlorite, the oxidation may also be performed with dilute chlorine or bromine water. If the urine contains albumin, the latter must first be precipitated by lead acetate and the test performed in the filtrate. If the urine is very dark, it may also be decolorized by precipitation with lead acetate.

2. Obermayer's Test.—As chloroform forms an emulsion with the urine that frequently renders it difficult to recognize the blue color of the indigo in chloroform solution, Obermayer first precipitates the urine with lead acetate, filters, oxidizes the indican in the filtrate with ferric chloride in concentrated hydrochloric acid, and then shakes out the indigo with chloroform. The blue solution then settles at the bottom of the tube. An excess of lead acetate must be avoided, otherwise lead chloride will precipitate when the hydrochloric acid is added, and this would render the chloroform cloudy. The addition of lead acetate has the additional advantage of removing bile pigment that may be present in the urine.

The filtrate from the lead-acetate precipitate is shaken with an equal volume of fuming hydrochloric acid containing 3 gm. of ferric chloride to the litre. The mixture should be agitated for about two minutes.

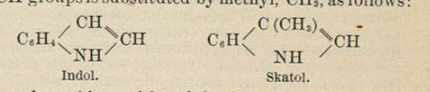
Quantitative Estimation of Indican.—Obermayer's test can be utilized for the quantitative determination. The urine, after treatment with Obermayer's reagent (3 gm. iron chloride to 1 litre of fuming hydrochloric acid) is repeatedly extracted with chloroform. In the chloroform solution the amount of indigo-blue can be determined: (*a*) by weighing, (*b*) colorimetrically, (*c*) spectrophotometrically.

(*a*) By Weighing.—The chloroform is driven off in a weighed dish and the residue dried at 110° C. and weighed.

(*b*) Colorimetrically.—The chloroform solution is compared with solutions of pure indigo-blue in chloroform of known percentage. By diluting the urinary chloroform extraction with measured quantities of chloroform until its color corresponds exactly to that of one of the standard tubes, the amount of indigo-blue in the urine can be estimated by a simple calculation.

(*c*) Spectrophotometrically (F. Müller).—This method requires much practice and a complicated apparatus, a spectrophotometer. I refer for a description of the method to Huppert, "Harnanalyse," p. 692 *ff.*). This method is not any more accurate than those given under (*a*) and (*b*).

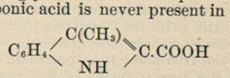
Skatoxyl. This body is an oxidation product of skatol, and skatol is indol in which one hydrogen atom of one of the CH groups is substituted by methyl, CH_3, as follows:



Skatoxyl combines with sulphuric acid, and appears in the urine as the sodium or potassium salt of skatoxyl-sulphuric acid. If more skatol is formed than can be combined with available sulphuric acid, the excess appears in the urine as skatoxyl glycuronic acid (see below). Skatol is a normal constituent of human fæces; whether it occurs normally in the urine remains doubtful for the present. This is due to the fact that the only qualitative index of the presence of skatol (skatoxyl) in the urine is the appearance of a red pigment when the indican tests are performed, and this red pigment may be indigo-red and due to the presence of indican. Skatoxyl sulphuric acid has been found in large quantities in cases of diabetes suffering from digestive disorders. It is possible that some of the many red pigments that have been found in the urine by many different observers are skatol derivatives.

The presence of skatol can be suspected if the urine turns dark on standing: the color usually becoming dark reddish or violet, or even black. If Jaffé's indican test is made the addition of hydrochloric acid alone will color the urine dark red or violet; nitric acid alone or with the addition of a little potassium nitrate produces a cherry-red color; ferric chloride with or without hydrochloric acid also a cherry-red color. The pigment separates out on boiling, and can be extracted with ether. These color tests unfortunately are not typical for skatol, for similar reactions are given by indol red. For order to differentiate the two the pigment must be precipitated from the urine, according to the method adopted in Jaffé's indican test; the pigment is then treated with zinc powder and indol or skatol liberated in this way. Numerous tests are given that are more or less typical for either body. If enough indol or skatol is present to yield a few crystals, the difference in the melting points may decide (indol 52° C., and skatol 95° C.). For other tests I refer to Huppert, "Harnanalyse," pp. 169 and 170).

Skatol can appear in the urine as skatoxyl sulphuric, skatoxyl glucuronic, and skatoxyl carbonic acid. The chemistry and formation of the former is analogous to that of indoxyl sulphuric acid, and much that has been said in regard to indol and its urinary congeners applies with equal force to skatol. Skatoxyl glycuronic acid forms if more skatoxyl is thrown into the circulation than can be combined with available sulphuric acid. This compound glycuronate will be presently discussed. Skatoxyl carbonic acid is never present in large quanti-



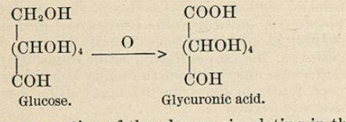
ties. It gives the ferric chloride reaction with hydrochloric acid or hypochlorite in the urine direct. In order to demonstrate its presence it must be isolated from the urine. As the clinical significance of this substance is not very great, the method of Salkowski (*Zeitschr. f. phys. Chem.*, vol. ix., 32) will not be described in this place.

Conjugate Glycuronates.—Glycuronic acid does not occur free in the urine, but only in combination with certain aromatic bodies, as phenol, paracresol, indoxyl, and

skatoxyl (see above), certain drugs, as naphthol, camphor, toluol, menthol, oil of turpentine, morphine, chloral, and many others. Some of these bodies appear only in combination with glycuronic acid, others, as the phenols, combined first with all the available sulphuric acid to conjugate sulphates, and with glycuronic acid, only after all the supply of sulphuric acid is exhausted. The resulting compounds partake of the character of glucosides.

The conjugate glycuronates are monobasic acids; all rotate the plane of polarized light to the left. The different members of this group, however, show individual peculiarities in other respects. By hydrolysis the glycuronates can be split into free glycuronic acid and the component alcohol. In some cases this disassociation occurs on gentle heating or even at ordinary temperatures, whereas in others boiling with dilute acids or heating with superheated steam is necessary. Some of the conjugate glycuronates reduce cupric oxide in alkaline solution; others do not. Some are precipitable by lead acetate and others are not. These differences are determined by the character of the alcohol in combination with the glycuronic acid molecule.

Glycuronic acid may be considered an oxidation product of glucose, the relation between the two being the following:



Glucose. Glycuronic acid.

As the greater portion of the glucose circulating in the blood is derived from the stored glycogen, it may be said that glycuronic acid is derived from glycogen *viâ* glucose. The acid must be formed in the tissues of the body, for if camphor or chloral, *e.g.*, are administered to a starving animal these bodies will appear in the urine as glycuronates. It is very probable, therefore, that glycuronic acid in this case is derived from the stored glycogen in the liver and muscles. If glycuronic acid, moreover, is fed to animals, the glycogen content of the liver is apparently spared, for after such feeding the liver glycogen increases. Another possible source of glycuronic acid is chondroitin-sulphuric acid (see under "Mucin"), for the latter can be split into glycuronic acid and glycosamin *viâ* chondrosin by a process of hydrolytic disassociation.

Free glycuronic acid and its salts rotate the plane of polarized light to the right, whereas, as we have seen, the aromatic glycuronates are levorotatory. As the free acid, its salts and esters, can reduce metal oxides, glycuronates may readily be taken for glucose. This confusion may be still more enhanced if phenylhydrazin compounds are made, for the free acid forms a crystalline phenylhydrazin compound that melts between 114° and 115° C. Glycuronic acid is not, however, fermentable.

Glycuronates will only be looked for in urine that is levorotatory. In order to demonstrate the presence of glycuronic acid, the latter must be liberated from its compounds. This can be done as follows:

Fifty cubic centimetres of the urine are treated with so much dilute sulphuric acid that the strength of the resulting mixture equals one per cent. The solution is heated over the free flame in a porcelain dish in order to bring about the disassociation of the various glycuronic acid compounds. Boiling for two or three minutes is sufficient. Without filtering, the *orcin test* is performed as follows: A saturated solution of orcin in concentrated hydrochloric acid containing a slight excess of orcin is the reagent. A few drops of this reagent are added to the above mixture and the solution boiled for one or two minutes; a red color should appear. If it does not appear after two minutes' boiling, it is useless to boil longer, for by prolonged boiling any glycuronic acid that might have been present would be decomposed. The orcin test can only be considered positive if certain characteristic absorption bands are found in the spectrum (see Salkowski: *Zeitschr. f. phys. Chem.*, 1899, No. 27).

In diabetes the appearance of glycuronic acid has attracted much attention of late years. As glycuronic acid is an oxidation product of glucose that may be considered intermediary between glucose and the terminal products of its disassimilation, viz., CO_2 and H_2O, the appearance of glycuronic acid in the urine of diabetics has been interpreted to signify incomplete oxidation of sugar. Several writers go so far as to claim that a "delayed Fehling test" in urine that is free from glucose but that contains glycuronic acid is evidence of deficient sugar destruction, and should be considered a warning of impending glycosuria and diabetes. This field is promising and the above hypothesis very seductive. The subject is not sufficiently worked out, however, to merit elaborate discussion in this place. I content myself with referring to it thus briefly. In looking for glycuronic acid in diabetic urine the sugar should first be removed by fermentation. If the liquid is levorotatory after fermentation, the presence of glycuronic acid or its compounds may be suspected; if it still reduces Fehling's or Nylander's solution, and if it gives the orcin reaction after boiling with one-per-cent. sulphuric acid, the existence of glycuronic acid may be considered demonstrated.

Compound Glycocolls.—Glycocoll is amido-acetic acid. It combines with certain aromatic decomposition products of the albumins that are formed in the intestinal tract by putrefaction or in the proper tissues by perverted katabolism—the resulting compounds appear in the urine as compound glycocolls. The two most important members of this group are *hippuric acid* and *phenaceturic acid*, the former a compound of phenylpropionic acid (after its oxidation to benzoic acid) with glycocoll, the latter a compound of phenylacetic acid with glycocoll.

Hippuric Acid. The source of the glycocoll entering into the composition of hippuric acid is obscure. It may be formed from albuminous putrefaction, as all albumins contain a glycocoll radical. The phenylpropionic acid and its oxidation product, benzoic acid, are derived from the degradation of albumins by intestinal putrefaction. In addition certain articles of diet—in particular, certain vegetables—contain bodies like cinnamic and quinic acid, toluol, etc., which may lead to the formation of benzoic acid. We find, therefore, that the elimination of hippuric acid is greater on a vegetable diet than on a meat diet, greater in herbivora than in carnivora. The excretion of hippuric acid in carnivora is exclusively due to the intestinal putrefaction of albumins. In man the ingestion of cranberries, plums, prunes, leads to an increased excretion of hippuric acid. The total hippuric-acid excretion may fluctuate from 0.1 to 1 gm. a day. The normal average excretion on an ordinary mixed diet containing more of the above-named fruits may be placed at 0.6 gm.

Test.—In order to demonstrate the presence of hippuric acid in the urine it is necessary to isolate it. The best method for the isolation of hippuric acid on a small scale is that recommended by Bunge and Schmiedeberg. About 500 c.c. of the urine are rendered alkaline with sodium carbonate, evaporated almost to dryness, and the residue repeatedly extracted with alcohol. The alcohol is driven off, the watery residue acidified with HCl and repeatedly extracted with acetic ether. The acetic ether extract is decanted off and washed with water, then evaporated at a low temperature. The residue contains hippuric acid and any benzoic acid or fat that may be present in the urine. If the residue is treated with petroleum ether, these impurities can be removed and pure hippuric acid remains behind. The hippuric acid is then crystallized from warm water at 55° C.

The crystals of hippuric acid can be recognized from their form, for they constitute milk-white, semitransparent, quadrilateral prisms and columns that are frequently united to form rosettes. On dry distillation (*i.e.*, on heating in a dry test tube) hippuric acid first melts to an

oily fluid (that again becomes crystalline on cooling), that gradually turns red if the temperature is raised sufficiently, and finally gives off benzoic acid that sublimes on the walls of the test tube and emits an agreeable odor at first of hay, later of prussic acid. The crystals of hippuric acid are very soluble in alcohol and hot water, and this property, too, may aid in identifying them. Confusion with phenaceturic acid (see below) can be avoided by performing a melting-point determination (melting point of hippuric acid 187.5° C.; melting point of phenaceturic acid 143° C.).

Phenaceturic Acid. This body is only rarely found in the urine of human subjects, but is common in the urine of horses and other herbivorous animals. It is derived from phenyl-acetic acid, a body that can arise from the intestinal putrefaction of albumin. It usually occurs together with hippuric acid, and can be separated from the latter, as shown above, by treating the mixture with petroleum ether. The acid crystallizes in rhombic plates with rounded angles. It is decomposed into phenylacetic acid and glycocoll by boiling with concentrated HCl. It assumes a red color when heated above its melting point (143° C.), and emits an aromatic odor.

Other Compound Glycocolls. In addition to hippuric and phenaceturic acid a number of other compound glycocolls have been found in the urine after the administration of certain drugs and chemicals that belong to the group of aromatic organic compounds. Thus salicylic acid appears in the urine as salicyluric acid, nitrobenzoic acid appears as nitrohippuric acid, furfurol appears as furfuracryluric acid, and p-toluylic acid as toluric acid, etc. Clinically these bodies are not very important, but chemically this synthesis to glycocoll compounds is very interesting.

Aromatic Oxyacids.—To this group belong para-oxyphenyl acetic acid, para-oxyphenyl-propionic acid, two bodies that are constantly present in human urine and that are derived from the intestinal putrefaction of albumin, or possibly, too, from proteid disassimilation in the tissues. A litre of normal human urine contains from 0.01 to 0.02 gm. of these acids. In acute phosphorus poisoning and after the administration of phenol or of tyrosin the urine may contain eight times as much as the normal quantity. These oxyacids either appear in the urine as such or as conjugate sulphates.

The clinical importance of these two oxyacids is so subordinate that their properties and the methods for isolating and identifying them will not be given in this article (see Huppert, "Harnanalyse," p. 237 *ff.*).

Other members of this group are para-oxyphenyl-lactoic and oxyamygdalic acid that may both appear after feeding tyrosin or in acute yellow atrophy of the liver where much tyrosin and leucin are thrown into the circulation. All the acids enumerated may, in fact, be considered (chemically) as derivatives of tyrosin.

The two members of this group that are clinically most interesting and most important are dioxyphenyl-acetic acid (*homogentisinic acid*) and trioxyphenyl-propionic acid (*uroleucinic acid*); the two are known by the name of *alkaptonic acids*.

The Alkaptonic Acids. Alkaptonuria.—Alkaptonic acids in solution turn dark on standing. This is also the most striking feature of alkaptonuric urine. Alkaptonuria is commonly believed to indicate abnormal intestinal putrefaction. It is possible that an abnormally large amount of tyrosin is formed that after absorption leads to the formation and excretion of alkaptonic acids. Feeding with tyrosin, as a matter of fact, leads to the excretion of hæmogentisinic acid. Alkaptonuria occasionally appears in several members of a family. Such individuals may be considered chemical (*scil.* metabolic) "sports." Alkaptonuria may persist for a lifetime without any disturbance of the individual's health. It may also appear in certain diseases that are accompanied by intestinal derangement. The excretion of alkaptonic acids is always greater on a meat diet than on a vegetable diet. Antifermentative drugs and laxatives exercise no effect on its excretion.

The alkaptonic acids reduce copper and silver salts, but they do not ferment with yeast, nor are they optically active. They both give a lemon-yellow precipitate with Millon's reagent (see above under Albumin) in the cold that gradually turns orange and, on heating, becomes brick-red.

Homogentisinic Acid. The separation of the two acids is clinically unimportant, for the recognition of alkaptonuria from the above urinary characteristics is sufficient for all purposes. The recognition of pyrocatechin and hydroquinone, that may also cause darkening of the urine, has already been discussed. The presence of these bodies should be ruled out.

For completeness sake, however, the following method for isolating the alkaptonic acids from the urine and for separating the two may be given:

To 1 litre of urine 150 c.c. of twelve-per-cent. H_2SO_4 are added and the mixture extracted with ether by repeatedly shaking it with renewed quantities. In this way nearly all the alkaptonic acids can be extracted. It is a good plan to concentrate the urine before extracting with ether, care being taken that the urine is acid while evaporating. The ether is driven off, the residue, which becomes crystalline on cooling, dissolved in a large volume of water, the solution heated almost to the boiling point and treated with a concentrated solution of lead acetate. The precipitate that forms is filtered off at once and the filtrate allowed to cool. From this solution the lead salt of homogentisinic acid crystallizes within twenty-four hours in the form of large prismatic crystals. In order to manufacture the free acid the lead salt is disassociated with a stream of sulphureted hydrogen, the lead sulphide filtered off, the filtrate freed from H_2S by heating, and concentrated on the water-bath until it begins to assume a dark color. The concentration is then continued *in vacuo* over H_2SO_4. The acid crystallizes in large crystals of characteristic shape that give off 9.08 per cent. of their weight at 100° and melt at 146.5° to 147° C. They are almost insoluble in chloroform, benzol, and toluol. Their watery solution gives the above-named reactions for alkaptonic acid, and turns blue with a few drops of ferric chloride.

Uroleucinic acid has been found only in one case of alkaptonuria. It can be differentiated from homogentisinic acid by the ferric chloride reaction, for whereas the solution of the former turns blue on addition of ferric chloride, the solution of the latter turns green. Its melting point is at 190° to 193° C. It gives the reactions common to the alkaptonic acids.

Fatty Acids.—*Volatile Fatty Acids.*—Normal urine always contains traces of volatile fatty acids, chiefly *formic*, *acetic*, *propionic*, and *butyric* acids. According to different estimations, from 0.04 to 0.06 gm. of volatile fatty acids are excreted in twenty-four hours. In febrile states this quantity is increased (0.17 gm.—chiefly acetic). When the urine undergoes ammoniacal decomposition, large quantities of these acids are formed, the source of these volatile acids in this case being the carbohydrates of the urine. Of other acids of the fatty series, both volatile and non-volatile, *valerianic* acid has been found in typhoid, variola, and acute atrophy of the liver. (In these states this acid is probably a putrefaction product of leucin.) In hæmatoporphyrinuria, and in a case of phosphorus poisoning, *oleic* acid and *solid fatty acids* (melting point 49° to 51° C.) have been recovered from the urine. In diabetic urine acetic, butyric, formic, and propionic acids have been found by different investigators. In the urine of various herbivorous animals particularly large quantities of this group of acids have been found. The bulk of the volatile fatty acids excreted in the urine is unquestionably derived from carbohydrate fermentation in the intestine. A portion may also be derived from the putrefaction of albumins in the bowel or from abnormal degradation of the tissue albumins.

Isolation and Identification. The urine (preferably large quantities) is acidified with phosphoric or sulphuric acid and distilled in a current of steam. The acid that is added converts the urea into carbonate of ammonia

and is bound by the latter. The distillation is continued as long as the distillate remains acid. The distillate is then neutralized with soda, evaporated to dryness, and the residue repeatedly extracted with absolute alcohol. The alcoholic solution contains sodium salts of the fatty acids, sodium benzoate, and traces of paracresol. The benzoic acid can be removed by treating the residue obtained from the evaporation of the alcoholic extract with sulphuric acid in the cold, removing the precipitate by filtration and allowing the filtrate to stand in the cold until all the benzoic acid is precipitated. The filtrate is again neutralized with soda and shaken with ether. This removes the paracresol. The ether is removed in a separating funnel, the watery residue heated to drive off the last traces of ether, and the analysis for fatty acids undertaken in the watery solution that remains behind.

For the identification of the different acids of this group I refer to text-books of organic chemistry. The total quantity of the fatty acids present in a stated quantity of urine can be determined by preparing the distillate as above, removing benzoic acid and paracresol as indicated, precipitating the watery solution of the fatty acids with barium hydrate, evaporating to dryness, weighing the residue of barium salts of the fatty acid, redissolving in water, determining the barium by precipitation with sulphuric acid, incinerating the barium sulphate and extracting the residue with hydrochloric acid and subtracting the weight of the barium from the weight of the barium salts of the fatty acids.

Lactic Acid (*Sarcolactic, paralactic, dextrorotary lactic acid*).—Lactic acid has been found in the urine in dyspnœa, in perversions of the liver function, after excessive muscular exercise and in certain affections of the muscles accompanied by disturbances of muscular nutrition (trichinosis), and finally in certain forms of poisoning. Normal urine never contains lactic acid. Paralactic acid in the form of its ammonia salt is probably a normal constituent of the blood entering the liver. In this organ it is converted into urea. Hence it appears in the urine when the urea-forming function of the liver is impaired, as in destruction of the liver parenchyma (phosphorus poisoning, acute yellow atrophy), and in asphyxia or carbon-monoxide poisoning when the available supply of oxygen is reduced. After epileptic seizures lactic acid has been found in the urine. Here its appearance may be explained either by the increased muscular catabolism incident to the spasm or by asphyxia incident to the tonic contractions of the diaphragm. Whether or not lactic acid is a disassimilation product of carbohydrates (*i.e.*, whether or not glycogen [dextrose] is destroyed to CO_2 and H_2O viâ lactic acid) remains for the present undetermined. In this case, too, a perversion of glycolysis, or excessive disassimilation of glycogen with lack of oxygen, might account for the appearance of lactic acid in the urine.

Isolation and Estimation. In order to estimate the quantity of lactic acid excreted the lactate of zinc must be manufactured as follows: The urine is treated with a large excess of phosphoric acid and repeatedly extracted with ether. The ethereal extract is evaporated. The residue constitutes a syrupy liquid containing any lactic acid that may be present—also phenols, urea, and hippuric acid. By dissolving the residue in water and boiling the liquid with lead hydrate the extraneous matter can be removed. The solution is filtered hot and the lead precipitated by a stream of H_2S. The sulphide of lead is filtered off, the filtrate boiled in order to drive off the H_2S, and then boiled again with carbonate of zinc. The solution is then evaporated to a small volume and allowed to crystallize, the crystals of zinc lactate washed with a little absolute alcohol, dried in the air and weighed. They are then heated in a small porcelain crucible to 100° to 110° C., and the loss of water of crystallization determined. The paralactate of zinc contains 14.58 per cent. of water of crystallization (ordinary racemose lactate of zinc 18.16 per cent). In addition a zinc determination must be made further to identify the compound. The dried and weighed salt is treated with concentrated nitric acid in a porcelain crucible, the HNO_3 driven off by gentle heat, and the residue first evaporated to dryness and then glowed. The substance at first turns brown, and is finally converted into white zinc oxide. This is glowed to a constant weight. From the zinc oxide the amount of zinc present in the quantity of zinc lactate employed for the analysis can easily be calculated. The dried lactate of zinc contains 33.43 per cent. of ZnO or 26.84 per cent. of Zn.

β-Oxybutyric Acid, Diacetic Acid, and Acetone.—The chemical relation existing between these three substances may be illustrated by the following scheme:

$$\underset{\beta\text{-Oxybutyric acid.}}{CH_3.CHOH.CH_2:COOH} + O = \underset{\text{Diacetic acid.}}{(CH_3CO).CH_2COOH}$$
$$= \underset{\text{Acetone.}}{CO(CH_3)_2} + CO_2$$

The three substances may appear in the urine together. Every urine containing oxybutyric acid contains diacetic acid, and usually acetone. It is possible that in process of determination the precursors of acetone are frequently converted into the latter body so that acetone may apparently appear alone. Diacetic acid may also appear without *β*-oxybutyric acid, but urine containing diacetic acid always contains acetone; at least for the reason just stated acetone is always detected in the distillate. This at least is the author's experience.

The source of *β*-oxybutyric acid and its congeners is not positively known. The preponderance of evidence speaks for an albuminous origin, although there are some facts on record that speak for a carbohydrate or a fat origin of these bodies.

β-oxybutyric acid never occurs in normal urine. It is common in diabetic urine. It has been found in scarlatina and measles, but in no other febrile disease. It has been reported in scurvy, in cacrinoma (coma), in uræmia, and in a case of hysteria. The acid usually appears in the form of its alkali or ammonia salts—occasionally free. There is no definite relation in diabetes between the excretion of sugar, ammonia, and *β*-oxybutyric acid.

Tests.—*β*-Oxybutyric acid should be looked for only in urine that gives the ferric-chloride test for diacetic acid (see below). Every urine, however, that gives this test does not necessarily contain *β*-oxybutyric acid (see above). If the urine is levorotatory (after removal of dextrose in diabetic urine by fermentation), the presence of *β*-oxybutyric acid is still more probable.

Method of Külz. If the urine gives the above tests, it should be evaporated to a syrupy consistence (diabetic urine after fermentation), treated with an equal volume of concentrated sulphuric acid and distilled. The distillate should be collected in a test tube. Cooling is unnecessary. If *β*-oxybutyric acid was present, the distillate will contain *α*-crotonic acid, and this body should crystallize on cooling the liquid.

$$\underset{\beta\text{-Oxybutyric acid.}}{CH_3.CH(OH).CH_2.COOH} = \underset{\alpha\text{-Crotonic acid.}}{CH_3.CH = CH.COOH} + H_2O$$

The mother liquid is removed from the crystals by pressing them between filter paper. The dry crystals should have a melting point of 71° to 72° C. If the urine contains only small quantities of *β*-oxybutyric acid, and if consequently the distillate contains only very little *α*-crotonic acid, the latter may not form crystals. The distillate should then be extracted with ether, the ethereal solution separated from the water in a separating funnel and evaporated to dryness. The melting point of the residue may then be determined.

For quantitative determinations one litre of the urine is extracted with alcohol, the alcoholic extract treated with sulphuric acid and ether, the ethereal extracts evaporated, the residue dissolved in a small quantity of water, the solution precipitated with lead acetate, the lead removed by H_2SO_4, the filtrate brought to a definite volume (20 to 25 c.c.) and submitted to polarimetric analysis (Coefficient $[a]_D = 20°$). (See also Wolpe, *Arch. f. exp. Path. u. Pharm.*, vol. xxi., p. 138, 1886.)

Diacetic Acid.—This body is common in diabetes, in malignant febrile diseases, during the eruptive stage of scarlatina and measles. It has been described in cholera, in gastric cancer, in many different digestive disorders, in inanition from starvation, and in persons living on an abundant animal diet. Diacetic acid, as shown above, often occurs together with *β*-oxybutyric acid and acetone. It may occur without the former, but it never occurs without the latter. As a matter of fact, normal urine always contains traces of acetone (see below).

Tests.—As diacetic acid is rapidly decomposed (within twenty-four hours at the latest), the specimens of urine should be fresh.

(1) Arnold's Test. This is the most useful clinical test, as it does not respond to acetone, nor to *β*-oxybutyric acid. It has the additional advantage that certain bodies (salicylic acid, antipyrin, phenols, acetates, skatoxyl sulphuric acid, thallin, etc.) that may be present in the urine and that respond to the popular iron chloride reaction, to be described presently, do not interfere with this test. The same applies to bile pigments that may be present. In order to make the color reaction more striking the urine may profitably be filtered through animal charcoal.

The reagents needed consist of a one-per-cent. solution of sodium nitrite and a solution of para-amido-acetophenone (1 gm. of para-amido-acetophenone dissolved in 100 c.c. of water is treated with HCl until the yellow solution becomes colorless); one part of the nitrite solution is mixed with two parts of the acetophenone solution. A small quantity of the urine is shaken with an equal volume of this reagent and then treated with a few drops of ammonia. An amorphous reddish-brown precipitate forms. This, however, does not alone denote the presence of diacetic acid. If the mixture, however, is treated with a large excess of concentrated hydrochloric acid (10 to 1), and if a violet-purplish color appears, the urine contains diacetic acid.

(2) The Ferric Chloride Reaction (Gerhardt's Test). The urine is treated with a ten-per-cent. solution of ferric chloride as long as a precipitate continues to form. After filtration (or without filtration) the liquid on addition of more ferric chloride should turn a Bordeaux-red. As other substances (see above) give the same reaction, the test is not quite reliable. The color produced with ferric chloride by most of these bodies can, however, be distinguished from the typical Bordeaux-red with a little practice. Salicylic acid produces a color that is more violet, the antipyretics a color that is more purple, and that, moreover, requires longer (two to three minutes) to appear. The diacetic acid color disappears very soon, whereas all the other colors remain permanently. The test may finally be modified in such a way that the diacetic acid is partially isolated from the urine by treating it with sulphuric acid until it is strongly acid and then extracting it with ether. The ethereal extract, when shaken with a small quantity of the ferric chloride solution, assumes the typical Bordeaux-red color that disappears again on boiling.

(3) Mörner's Test. The urine is treated with a little iodide of potassium and ferric-chloride solution in excess. On boiling, acetone-iodine(?) develops a substance that is very irritating to the mucous membranes. This test is as delicate as Gerhardt's test, but also ambiguous in the sense that the same reaction is obtained with urine containing acetone alone.

Quantitative Estimation. This can only be performed for diacetic acid and acetone together. See, therefore, quantitative estimation of acetone.

Acetone.—Normal urine always contains traces of acetone—usually about 0.001 gm. (7 to 10 mgm.). If the carbohydrates are withdrawn, the acetone excretion increases. In certain pathological conditions as much as 0.5 gm. of acetone may be excreted in twenty-four hours. The chief source of acetone are the tissue albumins. Increased catabolism of the body proteids and decreased oxidation lead to acetonuria. Whether or not urinary acetone is ever derived from intestinal fermentation of albumins remains undetermined. Acetonuria may occur in any of the following pathological conditions: Inanition from starvation, dyspnœa (asphyxia), poisoning with hæmolytic drugs, during narcosis, diabetes (here the amount of acetone excreted stands in no relation to the sugar—or the N-excretion); in hydrophobia, morphinism, phosphorus poisoning, many digestive disorders (autointoxications), in long-continued febrile diseases, and in eclampsia. It will be seen that acetonuria is a very common sign.

Tests. Legal's Test.—The urine may be treated with a few drops of a twenty-per-cent. solution of sodium nitroprusside and a small quantity of sodium hydrate solution. If acetone is present, the urine will turn red—this color rapidly fades; if a few drops of acetic acid are now added, the liquid becomes purple or violet-red. If no acetone is present, the liquid remains colorless even after the addition of acetic acid.

This is the only one of the acetone tests that can be performed with the urine itself. For all the other tests from 200 to 300 c.c. of urine, after the addition of a little phosphoric acid, must be distilled and the tests performed with the first 20 to 30 c.c. of the distillate that pass over.

Lieben's Iodoform Test.—A few cubic centimetres of the distillate are treated with a few drops of potassium hydrate solution and iodopotassic iodide added. If acetone is present, a yellow precipitate of iodoform will form, that consists of fine hexagonal plates and emits a characteristic odor of iodoform. This test is very delicate. Unfortunately a number of other bodies give the same test, notably alcohol and aldehydes.

Gunning has modified the test as follows in order to avoid this confusion with alcohols and aldehydes: He performs the test with an alcoholic iodine solution and ammonia. In the beginning a black precipitate of iodine-N develops that soon disappears, however, and brings the yellow crystalline deposit of iodoform to view. This test is not quite so sensitive as the original test of Lieben.

Reynolds' Test.—This test is based on the property of acetone to dissolve freshly precipitated mercuric oxide. The mercuric oxide is obtained by precipitating a solution of corrosive sublimate with an alcoholic potassium hydrate solution (yellow precipitate). Some of this mercuric oxide is added to the distillate and the mixture thoroughly shaken and filtered. The filtrate is superposed with a solution of ammonium sulphide. If acetone was present in the distillate, a black ring of mercuric sulphide will form at the plane of contact.

Quantitative Estimation. The Underlying Principle.—Acetone in alkaline solution is converted into iodoform by the action of iodopotassic iodide. The reaction proceeds in two stages that may be illustrated as follows:

$$(I_2 + KOH = KOI + HI)$$
$$(1)\ \underset{\text{Acetone.}}{CH_3.CO.CH_3} + 3KOI = \underset{\text{Tri-iodine-acetone.}}{CH_3.CO.CI_3} + 3KOH$$
$$(2)\ CH_3COCI_3 + KOH = \underset{\text{Acetate of potassium.}}{CH_3.COOK} + \underset{\text{Iodoform.}}{CHI_3}$$

The amount of iodine consumed in this reaction is determined by titrating with sodium thiosulphate. The chemism of this reaction is complicated. I will limit myself to describing the execution of the method. An exhaustive explanation of the reaction will be found in text-books of chemistry.

Execution: The urine is distilled and the distillate must be free from phenols, ammonia, nitrous acid or formic acid, for all these bodies interfere with the titration or the formation of iodoform. The ammonia can be kept back by the addition of an acid; 2 c.c. of fifty-per-cent. acetic acid to 100 c.c. of urine do not liberate phenols (as stronger acids or more acid would do), but this amount allows a little ammonia to escape. The distillate should, therefore, be distilled a second time with 1 c.c. of twelve-per-cent. sulphuric acid. In order to prevent the passage of nitrous acid and of formic acid a little calcium carbonate is added to the urine prior to distillation.

To the second distillate is added an excess of a one-tenth normal iodine solution (12.685 gm. of iodine to 1,000 c.c.) and then drop by drop so much of a fifty-per-cent. solution of sodium hydrate that the color of the iodine disappears completely and the yellow iodoform precipitate appears. The mixture is thoroughly shaken and allowed to stand for five minutes. The liquid is now acidified with concentrated hydrochloric acid. The fluid turns brown from the iodine in excess that is liberated. This excess is titrated with a solution of sodium thiosulphate (one-tenth normal, *i.e.*, containing 24.8 gm. of the salt to 1,000 c.c. of water), using starch solution as an indicator. The starch should not be added until the liquid has become faint yellow on addition of a sufficient quantity of the thiosulphate. As soon as the mixture turns blue, the thiosulphate is slowly added drop by drop until finally the blue color disappears. Every cubic centimetre of the sodium thiosulphate solution corresponds to a cubic centimetre of the iodine solution. The number of cubic centimetres of the former employed in the titration can, therefore, be subtracted from the amount of iodine solution added to the distillate in order to give the amount of iodine consumed in the reaction. The figure obtained multiplied by 0.967 indicates the number of milligrams of acetone present in the amount of urine employed for the estimation.

Pigments.—Indican, indigo-red and skatol-red, alkaptone, pyrocatechin, hydroquinone have already been discussed. The rare or clinically unimportant pigments urochrome, uromelanin, humin bodies, uroerythrin, urorosein, urorubrohæmatin, urofuscohæmatin, and the large number of undefined urinary pigments cannot be described in this article. The following urinary pigments are important: (1) The bile pigments (and bile acids); (2) urobilin; (3) melanin; (4) hæmatin; (5) hæmatoporphyrin.

The Bile Pigments (and Bile Acids) (Choluria).—(*a*) *Bile pigments* are never found in normal urine. They appear in the urine whenever there is stasis of bile and diapedesis of bilirubin from the bile channels into the lymph spaces and blood-vessels of the liver ("hepatogenous" icterus). Thus choluria is one of the signs of occlusion or stenosis of bile ducts, impairment of the diaphragmatic excursions, thrombosis of the portal vein, etc. Some forms of choluria are due to so-called "hæmatogenous" icterus, *i.e.*, the development of bile pigments from the blood pigment of extravasations, hæmatomata, etc. The writer has shown that bile pigments can be formed outside of the liver whenever hæmoglobin undergoes disintegration in the tissues. The appearance of bile pigment in the urine by no means, therefore, always indicates an affection of the liver or of its ducts and vessels.

Urine containing bile pigment is usually yellowish or greenish-brown. The foam that forms on shaking the urine is usually yellow, even if the urine only contains very minute quantities of pigment. The only bile pigment that is found in freshly voided urine is bilirubin. The other bile pigments—viz., biliverdin, bilifuscin, and biliprasin—develop secondarily from bilirubin if the urine is allowed to stand. All the bile pigment voided in the urine may occasionally be found in the sediment. The differentiation of the different bile pigments is clinically unimportant. Of the many chemical tests for bile pigment only the two most practical ones will be described.

(1) Gmelin's Test.—A few cubic centimetres of yellow nitric acid are superposed in a test tube with an equal volume of urine. If bile pigment is present, a *green* ring will form at the plane of contact, in addition usually to a number of other multicolored zones. Only the *green* color is characteristic for bile pigment (biliverdin). The urine must be free from alcohol, as this substance also gives a bluish-green ring with nitric acid. Rosenbach has described a convenient modification of the test. A piece of filter paper is moistened with the urine to be tested and a drop of yellow nitric acid placed on the paper; a number of concentric colored rings form around the drop, and again only a green ring is characteristic for bile pigment. Large quantities of albumin in the presence of small quantities of pigment interfere with Gmelin's test; they had better, therefore, be removed. Small quantities of albumin in the presence of large quantities of pigment do not interfere.

(2) Huppert's Test for very Small Quantities of Bile Pigment.—Ten cubic centimetres of the urine are treated with lime water. The precipitate is removed by filtration and brought into a test tube containing sulphuric-acid alcohol. The suspension is acidified with a few drops of sulphuric acid and heated to boiling. If bile pigment is present, the liquid turns green. Again, the green color is important; indican may give a yellowish or reddish color, but only bile pigment gives a green tint to the alcoholic solution.

(*b*) *Bile acids* in the urine are always pathological. They usually appear together with bile pigment and possess the same clinical significance (see above). It is useless to attempt the direct demonstration of bile aids in the urine, as the most typical reaction for bile acids, viz., Pettenkofer's reaction, is given by a number of other normal ingredients of the urine. The bile acids must, therefore, first be isolated as follows: Albumin, if present, is removed by coagulation; the coagulate filtered off and the filtrate evaporated to dryness, the residue extracted with absolute alcohol, the alcoholic extract diluted with water and precipitated with basic lead acetate and ammonia, the precipitate filtered off, extracted with absolute alcohol and filtered hot. This solution contains any bile-acid salts that may be present and can be submitted to the following tests:

(1) Pettenkofer's Reaction (modified by Udranszky).—In the original Pettenkofer test cane-sugar and sulphuric acid were employed. Since it was shown that the sugar was converted into *furfurol* by the action of the sulphuric acid and that the furfurol gave rise to the typical color reaction with bile acid, Udranszky advised the use of furfurol in the first place. One cubic centimetre of the bile-acid solution is treated with one drop of a one-tenth-per-cent. solution of furfurol and slowly superposed upon 1 c.c. of concentrated sulphuric acid, care being taken by immersing the tube in cold water to prevent too great heating of the mixture. A purple color appears at the plane of contact that gradually extends upward into the superposed solution; on standing, the color turns bluish. In alcoholic solution a green fluorescence is seen. The pigment gives a typical spectrum, viz., two bands, the one near F, the other between D and E, near E.

(2) Platner's Crystals.—An attempt may be made to obtain crystals of the bile acids by evaporating the solution with soda, extracting the residue with absolute alcohol, and adding enough ether to produce a slight clouding. On standing, Platner's crystals of the sodium salts of the bile acids form. These may further be identified as above or by testing their effect on the heart of a curarized frog (retardation).

Urobilin.—Normal urine never contains urobilin. It does, however, contain the chromogen of urobilin, viz., urobilinogen, from which urobilin develops by the action of sunlight. Only very minute quantities of urobilin (urobilinogen) are found in normal urine (not more than from 20 to 130 mgm. in the quantity passed by a healthy adult in twenty-four hours). The high figure (130 mgm.) is only obtained if the urine is first allowed to stand so that all the urobilinogen it contains is converted into urobilin. Urobilin is produced in the bowel by putrefaction, *i.e.*, directly by the action of reducing bacteria on bile pigments. Consequently the excretion of urobilin is increased if intestinal putrefaction is great. It is absent from the urine of the new-born (first to third day) because the intestinal contents remain sterile for some time. It is also absent from the urine if the entrance of bile into the intestine is prevented (occlusion of the bile ducts as by carcinoma of the gall bladder or pancreas, catarrhal swelling, etc.). In these diseases, therefore, the *absence* of urobilin from the urine may be of diagnostic value. *Urobilin is increased* whenever hæmolysis occurs

(even if the bile ducts should be occluded at the same time—urobilin-icterus). I propose the name of "*hæmatogenous urobilinuria*" for this condition. Thus we find urobilinuria in many infectious diseases, particularly those forms that are accompanied by rapidly progressive anæmia, in poisoning with hæmolytic substances (potassium chlorate, mushrooms, etc.), in absorption of hemorrhagic exudates, effusions, cysts, etc.

Immediately after the removal of some biliary obturator (passage of a gall-stone, relief of catarrh, etc.) the urobilin excretion is increased—a direct result of the entrance of much bile into the bowel. Urobilin has also been found increased in pernicious anæmia, after chloroform narcosis, in a case of melanotic sarcoma (alternating with melanin), in lead colic, atrophic cirrhosis of the liver, and in certain valvular heart lesions that lead to passive congestion of the liver and pleiochromia.

Tests.—Urine containing much urobilin is usually very dark. The color is no index, however, of the amount of urobilin, for very dark urines may be colored by other ingredients, and may contain very little urobilin, whereas light urines may contain much urobilin, as the coloring properties of urobilin are slight. Sometimes the presence of urobilin can be demonstrated in the urine direct. Occasionally it may be necessary to isolate it from the urine.

To test the urine direct we have two tests, viz.:

(1) Fluorescence. The urine is treated with an excess of ammonia and a few drops of a one-per-cent. alcoholic solution of chloride of zinc. If the urine contains urobilin, a beautiful green fluorescence will appear.

(2) Spectroscopic Examination. Only such urine can be utilized for this test that does not contain too many other pigments. It is well, therefore, to treat the urine first with mercuric sulphate, to filter off the pigment precipitate, and to examine the filtrate spectroscopically. Ten cubic centimetres of the urine are treated with 5 c.c. of a solution of mercuric sulphate, made by dissolving 5 gm. of mercuric oxide in 20 c.c. of H_2SO_4 and 100 c.c. of water; the mixture is allowed to stand for five minutes, then filtered. The filtrate should show the absorption bands of mercuri-urobilin. The urine itself and extracts made from the urine show either the spectrum of urobilin itself or of acid urobilin. (For descriptions and illustrations of these different spectra I must refer to text-books of physiological chemistry.)

If the urine contains too little urobilin to give these tests positively, the urobilin should be extracted. One may use amyl alcohol, chlorform, phenol, ether, or acetic ether. Amy alcohol is the best.

Extraction with Amyl Alcohol (Nencki and Sieber). Ten to twenty cubic centimetres of the urine are treated with a few drops of hydrochloric acid and shaken with 6 to 10 c.c. of amyl alcohol. The alcoholic solution is submitted to spectroscopic analysis. It is then treated with a few drops of a one-per-cent. solution of zinc chloride in ammonia-alcohol. In the presence of urobilin a green fluorescence appears.

Melanin (Syn. *Phymatorhusin*).—Patients with melanotic neoplasm occasionally void urine that turns dark on standing. The dark color can frequently be brought about more rapidly by the addition to the urine of oxidizing agents, as nitric acid, chromic acid, ferric chloride, bromine, etc. The dark color may occasionally be made to disappear again if the urine is treated with reducing agents. If the urine is kept in an air-tight vessel, darkening may be prevented. Indican and urobilinogen may lead to confusion. This error can only be ruled out by testing the urine for these two bodies. After their presence is excluded the presence of melanin can be established by the properties of melanotic urine, described above.

Hæmatin is occasionally found in the urine. It is a derivative of hæmoglobin and may be formed from the latter if bloody urine is heated, the urinary acids disintegrating the hæmoglobin into hæmatin and a proteid radical. It has also been described in a case of sulphuric-acid poisoning. The presence of hæmatin is recognized by spectroscopic examination of the urine. The spectrum of hæmatin may be confused with the spectrum of methæmoglobin; hence it is necessary to treat the urine first with ammonia and to re-examine the filtrate spectroscopically; finally to add ammonium sulphide and to examine a third time. Spectra that are characteristic for alkaline and for reduced hæmatin will successively appear on the addition of these reagents. As this body is clinically unimportant, other tests and reactions will not be included in this article.

Hæmatoporphyrin.—This pigment in very minute traces occurs in normal urine (Garrod and Saillet). In a number of cases of enteric fever, Addison's disease, exophthalmic goitre, in cirrhosis of the liver, in anæmia, in lead poisoning, rheumatism, and gout considerable quantities of hæmatoporphyrin have been found in the urine. The source of hæmatoporphyrin is obscure. It does not seem to occur regularly whenever many red blood corpuscles are destroyed. The theory that it is formed in the bowel from blood pigment has been exploded.

The most important clinical forms of hæmatoporphyrinuria occur in poisoning with sulphonal, trional, and tetronal, or after the prolonged use of these drugs. The author has reported a case of sulphonal poisoning with hæmatoporphyrinuria (together with J. Tyson) in which 1.683, 1.013, and 0.098 gm. of hæmatoporphyrin were voided on three successive days, this amount corresponding to 35.1 gm. of hæmoglobin. Here about one-seventeenth of the total hæmoglobin of the subject (computed at 600 gm.) was destroyed and appeared in the urine as hæmatoporphyrin. Salkowski in one case of sulphonal poisoning obtained 0.87 gm. of hæmatoporphyrin.

Tests and Determination.—Hæmatoporphyrin urine has a typical Burgundy-red color. On standing it may turn almost black. It is possible that such urine contains other pigments besides hæmatoporphyrin. The urine is precipitated with barium mixture (consisting of one part of saturated barium-nitrate solution and two parts of concentrated baryta water). The precipitate contains the bulk of the hæmatoporphyrin together with other pigments that may be present. The precipitate is filtered off, washed and extracted with dilute hydrochloric-acid alcohol. This extract is reddish or pink in color, fluoresces and turns darker on heating. Spectroscopically a narrow absorption band between C and D in the yellow and a second broader band between D and E between the yellow and the green will be found. If the solution is alkalinized four bands will appear, one between C and D, two between D and E, and a fourth very dark band between *b* and F, *i.e.*, between the green and the blue.

Quantitative Estimation.—One hundred cubic centimetres of the urine are rendered alkaline with a dilute soda solution and precipitated with calcium chloride, again rendered alkaline with soda and the precipitate filtered off. The dark-red precipitate is repeatedly washed with water until the filtrates are free from chlorine (no precipitate with $AgNO_3$), then washed with absolute alcohol, and finally extracted with dilute HCl-alcohol at a temperature of about 40° C. From the pink alcoholic extract the pigment can be precipitated with water after neutralization of the liquid with dilute ammonia. The flocculent precipitate is gathered on a filter, washed free from chloride, washed with alcohol and ether, desiccated *in vacuo*, dried at 115° C., and weighed. This precipitate, besides hæmatoporphyrin, contains inorganic salts. A weighed portion is therefore incinerated and the weight of the ash subtracted. The difference represents organic substance, *i.e.*, hæmatoporphyrin.

Fat.—For fat in lymph cells, epithelia, casts, see section on "Urinary Sediments"; for fat concretions see section on "Urinary Concretions"; for fat in chyluria, see the article on this subject.

Cholesterin has been found in chyluria (see article on this subject). It occasionally appears in the urine of pregnant women, and it has been found in the urine of

epileptics. For cholesterin in urinary concretions and sediments see below.

Tests.—The urine is shaken with ether, the ethereal extract removed, the ether evaporated, and the residue examined microscopically for crystals of cholesterin (see Fig. 4869). If the residue contains so much fat that the cholesterin cannot crystallize or the crystals cannot be recognized, the residue is treated with alcoholic KOH, the mixture boiled, evaporated to dryness, the soaps that have been formed dissolved in water, the watery solution extracted with ether, the ether evaporated, the residue dissolved in boiling alcohol, the solution evaporated to a small volume and allowed to crystallize. Cholesterin crystals touched with concentrated sulphuric acid turn red or blue (cholesterilines).

Inosite (Hexahydro-hexoxybenzol).—This body is occasionally found in diabetes mellitus, in albuminuria, and after abundant water drinking. Pohl reports a case of diabetes mellitus in which the dextrose of the urine was gradually replaced by inosite. Inosite has been called a sugar, but it does not give the chemical reactions of any of the saccharides. It is, moreover, according to Maguenne hexahydroxybenzol. In order to demonstrate the presence of inosite it must be isolated from the urine. The best method is that of

Cooper-Lane.—The urine, after removal of any albumin it may contain, is precipitated with baryta water, filtered, and the washed filtrate treated with lead acetate until no further precipitation occurs. The lead precipitate is filtered off after twelve hours' standing, suspended in water and disassociated with H_2S. The lead-free filtrate is concentrated and treated with boiling alcohol (three to four volumes). The alcohol produces a precipitate that is removed by filtration; the filtrate is allowed to stand twenty-four hours; crystals of inosite may form. These are removed by filtration, washed with cold alcohol and identified (see below). If no crystals form, the alcoholic solution is treated with ether until the liquid becomes milky. The mixture after standing twenty-four hours precipitates fine shiny plates of inosite. The crystals give the following reactions:

(1) A few crystals are heated on a piece of platinum foil with a few drops of concentrated nitric acid and the mixture evaporated to dryness; the residue is treated with ammonia and a little $CaCl_2$ solution (to bind the excess of HNO_3) and again carefully evaporated to dryness; a bright rose color indicates the presence of inosite. One milligram of the substance gives this reaction distinctly. Carbohydrates do not give it.

(2) The water of crystallization may be determined in the crystals. At 100° C. the crystals should lose 16.67 per cent. of water.

(3) Inosite tastes sweet. It melts at 217° C. It crystallizes in clino-rhomboid crystals that are usually gathered together in rosettes.

Ptomaïns.—That urine is toxic has been known for a long time. The chief cause of this toxicity are the potassium salts the urine incorporates. After removal of the K-salts the urine, however, still remains toxic. The toxicity of the urine, moreover, is not proportionate to the quantity of K-salts it contains, and the urinary ash, finally, containing all the K-salts is not so toxic as the total urine. Bouchard and his pupils are the chief exponents of the toxicity of the urine. They have published a mass of investigations on the toxicity of the urine in health and disease. The results obtained from this work are out of all proportion to the labor expended; nothing really conclusive having so far been demonstrated. This is not the place to review this large field. It may suffice to say that a variety of organic bases, alkaloids and toxalbumins, have been found by different investigators in normal and in pathological urines.

The best method for isolating ptomaïns from the urine is that described by Brieger:

Ten to twenty litres of the urine are acidified with HCl and evaporated to a syrupy consistence; the residue is extracted with alcohol and the solution precipitated with an alcoholic solution of mercuric chloride. The precipitate is extracted with much boiling water; the mercury compounds of the ptomaïns will then be found in the solution. The mercury compounds are disassociated with H_2S, the mercuric sulphide filtered off and the filtrate evaporated to a small volume. From this liquid the bases crystallize in the form of the hydrochlorates.

Enzymes.—The urine seems normally to contain proteolytic and diastatic ferments. Of the former the most common one can digest fibrin in dilute HCl solutions. It appears, therefore, to be pepsin. Another protease is occasionally found that digests fibrin in an alkaline medium. It may be trypsin. A diastatic ferment (ptyalin) is apparently one of the constant constituents of normal urine. Rennet, too, has occasionally been found. Fat-splitting enzymes have so far not been positively demonstrated. It is probable that they would be destroyed in the bladder even if they were excreted in the kidneys and would consequently escape detection. The enzyme excretion fluctuates somewhat according to time of day, the time of eating, physical exercise, etc. So far similar fluctuations that a number of authors have described in various diseases have no clinical significance. For methods to identify the different enzmyes I refer to text-books of physiological chemistry.

Inorganic Constituents.

The inorganic constituents normally present in the urine are hydrochloric, sulphuric, phosphoric, and carbonic acids; potassium, sodium, ammonium, calcium, and magnesium. Other acids occurring in very small amounts are fluoric, silicic, nitric, and nitrous acids. In addition we sometimes find peroxide of hydrogen and sulphureted hydrogen, and, finally, of the bases, iron.

Urinary Ash.—The amount of urinary ash fluctuates from 9 to 25 gm. in the twenty-four hours' quantity; the chief fluctuations being due to variations in the sodium-chloride excretion.

Quantitative Estimation of the Urinary Ash.—In determining the total ash of a given quantity of urine, the urine after evaporation to dryness should not be directly incinerated, for the alkaline chlorides are volatile at glow temperatures; the sulphates are reduced to sulphides by carbonaceous material, and the monophosphates are decomposed (escape of phosphorus). The urine is therefore evaporated and the residue merely carbonified, not incinerated, at a low temperature; the soluble salts leached out with water, the residue dried and then incinerated, and, finally, the watery extract evaporated to dryness in the crucible containing the ash and the whole weighed. This method gives very accurate results. See also "Electric Conductivity of the Urine."

Acids.—1. *Hydrochloric Acid* (Chlorides).—The quantity of HCl in the urine is largely dependent on the ingestion of salt (NaCl). The daily excretion rarely exceeds an amount that would correspond to more than 15 gm. of NaCl. The excretion of HCl salts is greatly diminished in fasting and in pneumonia during the formation of the exudate. In nephritis, with retention of solids and in certain gastric disorders (hyperchlorhydria) the urinary chlorides are also decreased. After large meals containing much salt, during the absorption of fluid transudates, in interstitial hepatitis (?), after the administration of thyroid, after inhalation of chloroform, and in the stage of convalescence from acute nephritis, the urinary chloride excretion is increased. Hydrochloric acid appears in the urine as sodium, potassium, ammonium, calcium, and magnesium chloride. Of these salts the most important and the most abundant is the sodium salt. All these chlorides are readily soluble in water.

Tests.—The urine is treated with nitric acid and a few drops of a ten-per-cent. silver-nitrate solution. The precipitation of a cheesy sediment that is readily soluble in an excess of ammonia indicates the presence of chlorides. Other substances that react similarly are iodides, bromides, and cyanides. Their presence should, therefore, be excluded in interpreting this test.

Quantitative Estimation of the Chlorides.—1. Method of Volhard. Solutions: (1) 29.042 gm. of chemically pure silver nitrate dissolved in 1,000 c.c. of water.

(2) Concentrated solution of ferric-ammonium alum (free from chlorine!).

(3) Nitric acid of 1.200 specific gravity.

(4) Solution of ammonium-sulphocyanide. This solution should be of such a strength that 25 c.c. would correspond to 10 c.c. of the silver solution. Theoretically there should be 12.984 gm. of the salt to a litre, but as ammonium sulphocyanide is very hygroscopic, it cannot be weighed accurately. Consequently the strength of the solution must be determined by titrating against the silver solution: 6 to 7 gm. of the sulphocyanide are dissolved in 400 c.c. of water and a part poured into a burette; 10 c.c. of the silver solution diluted with 100 c.c. of water are poured into a flask treated with 4 c.c. of the nitric-acid solution, and 5 c.c. of the ferric-ammonium solution, and the whole thoroughly mixed by shaking. To the mixture so much of the sulphocyanide solution is allowed to flow from the burette that the liquid in the flask assumes a faint but permanent reddish tint. Several tests of this kind are made and the average quantity of the sulphocyanide solution required determined. If it is found, for instance, that 22 c.c. of sulphocyanide produce the end reaction (red color), then the volume of water that must be added to one litre of the sulphocyanide solution is found according to the formula: $22:25 = 1{,}000 : x$; $x = 1136.3$. In other words, in order that 25 c.c. of the sulphocyanide solution correspond to 10 c.c. of the silver-nitrate solution, 136.3 of water must be added to the sulphocyanide solution. With these four solutions the chloride determination in the urine is performed as follows: 10 c.c. of the urine are poured into a flask holding 100 c.c.; to the urine are added 50 c.c. of water, 4 c.c. of the nitric acid, and 15 c.c. of the silver-nitrate solution. The contents of the flask are agitated until the precipitate settles and the supernatant fluid becomes clear. The flask is now filled to a mark that indicates 100 c.c.; 80 c.c. of the contents are then filtered through a dry filter into a dry measuring cylinder. These 80 c.c. are poured into a flask holding about 200 c.c. and are treated with 5 c.c. of the ferric-ammonium-alum solution, and with so much of the sulphocyanide solution, prepared as above (from a burette), that a faint red hue persists. The amount of sulphocyanide solution required to bring about this end reaction is determined in a series of tests and the average taken. It is assumed that the 15 c.c. of silver-nitrate solution suffice not only to precipitate all the chlorides, but also to leave an excess of silver nitrate in solution. It is this latter excess precisely that is now determined by titration with the sulphocyanide solution. The percentage of chlorides in the urine is then calculated from the deficit, according to the following formula:

$$x = (37.5 - \tfrac{5}{4} R), \tfrac{1}{10}$$

x = Amount of sodium chloride contained in one litre of urine (expressed in grams).
R = Number of cubic centimetres of the sulphocyanide solution required to bring about the end reaction.

The rationale of this formula is the following: 10 c.c. of the silver-nitrate solution correspond to 25 c.c. of the sulphocyanide solution, and 15 c.c. of the silver-nitrate solution consequently correspond to 37.5 c.c. of the sulphocyanide solution. For 100 c.c. of the test solution 37.5 c.c. of the sulphocyanide solution less $\frac{5}{4}$ of the amount of sulphocyanide solution used were needed, for 80 c.c. correspond to the amount of sulphocyanide solution taken from the burette, so that 100 c.c. would have required $\frac{5}{4}$ of this amount. As 25 c.c. of the sulphocyanide solution correspond to 10 c.c. of the silver solution, 1 c.c. of the former solution corresponds to 0.4 c.c. of the latter; and as 1 c.c. of silver solution indicates 0.01 gm. of sodium chloride (the silver solution used as a reagent is prepared in this proportion), 0.4 c.c. of the silver solution correspond to 0.004 gm. of sodium chloride. Hence in order to obtain the amount of chlorides present in 10 c.c. of urine the figure $(37.5 - \frac{5}{4} R)$ must be multiplied by 0.004. In order to determine the chlorides in one litre (1,000 c.c.) of urine the above figure must be multiplied by $0.4 = \frac{4}{10}$. (von Jaksch.)

The presence of albumin and sugar in the urine does not interfere with the accuracy of this method. It is the most accurate and the most convenient one for clinical purposes. All other methods, *i.e.*, the methods of Mohr, of Gay Lussac, of Zuelzer, are based on the same principle, viz., the titration of the chlorine with a solution of silver nitrate. As they have no advantages over Volhard's method, they will not be described in this place. A description will be found in Huppert, "Analyse des Harns," 1898, p. 705 *ff.*

Hydrofluoric Acid.—The urine contains traces of hydrofluoric acid (salts). It is clinically without any importance.

Test (*Berzelius*).—A large volume of urine is precipitated with ammonia, the precipitate calcined, treated with an equal volume of H_2SO_4 and heated in a platinum crucible. Over the crucible a glass plate is held. If HFl is present, it will etch the glass.

Sulphuric Acid (*Sulphates*).—Sulphates occur in the urine as mineral (preformed) and conjugate sulphates. For the latter see the chapter on Conjugate Sulphates above. The total SO_3 excretion fluctuates from 1.5 to 3 gm. in twenty-four hours on a mixed diet. The chief source of the sulphates are the albumins; the greater, therefore, the percentage of proteid pabulum, the greater the sulphate excretion. The conjugate sulphates constitute about one-tenth of the total sulphates. In certain pathological conditions the excretion of conjugate (ethereal) sulphates is greatly increased (see above), so that this proportion is changed in favor of the conjugate sulphates. In addition to preformed and conjugate sulphates the urine also contains sulphur in the form of sulphocyanides, thiosulphates, and sulphureted hydrogen, so-called "neutral sulphur" (see below).

The preformed sulphates of the urine are the sodium, potassium, magnesium, and calcium sulphate. The absolute increase or decrease of the preformed sulphates is of slight clinical significance. It is important, however, as pointed out above, to determine the relative proportion of preformed and conjugate sulphates; hence the quantitative estimation of the preformed sulphates is often necessary.

Qualitative Determination of Preformed Sulphates.—The urine is acidified with acetic acid and treated with barium chloride. A white, fine, powdery precipitate indicates the presence of preformed sulphates. This reaction is always obtained in normal urine. If the urine is not rendered sufficiently acid, the barium chloride may also precipitate phosphates or oxalates (or even uric acid). In order to differentiate the sulphates from these other bodies the precipitate is warmed with dilute HCl. This leads to the solution of oxalates and phosphates and leaves the sulphates behind.

Quantitative Determination of Preformed Sulphates. The preformed sulphates can either be determined directly or the total sulphates and the conjugate sulphates can be determined separately and the preformed sulphates calculated from the difference.

Total Sulphates.—The urine is treated with concentrated HCl and heated to boiling. This decomposes the conjugate sulphates into aromatic constituents and sulphuric acid. In the liquid the sulphates are precipitated with barium chloride, filtered off, and determined as barium sulphate.

Fifty cubic centimetres of urine are diluted one-half and treated with 5 to 10 c.c. of strong HCl heated to boiling and precipitated with a slight excess of barium chloride. The mixture is allowed to stand for twenty-four hours in the cold and filtered; the precipitate is washed with hot water, alcohol, and ether, and dried. The filter is then incinerated and weighed. One hundred parts of barium sulphate correspond to 34.28 parts of SO_3, or 41.13 parts of SO_4, or 41.99 parts of H_2SO_4, and the result may, therefore, be expressed as SO_3, or