tion of proteid pabulum. In interpreting the plus or minus of urea excretion both factors must nevertheless be included in the calculation, for it is clear that a diseased person who is consuming an abnormal quantity of the organized albumin of his proper tissues, and at the same time is eating a normal amount of proteid food, will excrete more urea than a healthy individual who is destroying a normal amount of body tissues and is eating the same amount of food. Or, again, a healthy, underfed subject will excrete less urea than an underfed, diseased subject, for the latter contributes more to the urinary nitrogen from his own tissues.

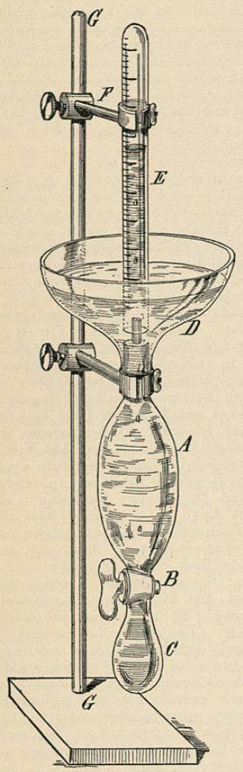

FIG. 4856.—Hüfner's Apparatus.

Normal urine contains as much as four per cent. of urea. The average quantity excreted in twenty-four hours by a healthy adult on a mixed diet is 33 gm. Fluctuations in the urea excretions from 20 to 45 gm. may nevertheless still be within physiological limits; a person performing hard physical labor will excrete more than 33 gm.; a person leading a sedentary life less. On a diet containing little N or after fasting the urea excretion may fall to 15 to 20 gm. Women excrete from 20 to 32 gm. a day. On a diet rich in N as much as 100 gm. may be excreted. The excretion of urea is always increased in febrile diseases, and in diabetes mellitus. It is always decreased in affections of the liver and kidney parenchyma, and in all chronic disorders that are accompanied by malnutrition.

Tests. Qualitative tests for urea are of no clinical importance. Quantitative estimations of the urea excretion, on the other hand, are of great clinical interest as an index of a variety of functional and organic perversions that have been indicated above.

Estimation of the Urea from the Specific Gravity of the Urine.—As urea is the chief solid constituent of the urine, the urea content can be approximately estimated from the specific gravity. Empirically it has been determined that urine of a specific gravity of 1.014 contains about one per cent. of urea; of 1.014 to 1.020, about one and a half per cent.; of 1.020 to 1.024, about two to two and a half per cent.; of 1.028, about three per cent. of urea. In febrile and cachectic states, in which the urinary chlorides are reduced, this estimation must be corrected. For the chlorides in these diseases are excreted in such small amounts that they exercise no appreciable influence on the urinary specific gravity. Consequently, a given specific gravity indicates *more* urea than is given in the above figures.

Of the accurate methods for determining urea the method of Hüfner is the most convenient for clinical work, the method of Mörner and Sjöquist the most accurate for scientific work. These two methods alone will therefore be described. The titration method of Liebig-Pflüger, the method of Folin, etc., are all very good, but they do not surpass in convenience nor in accuracy the two methods mentioned and to be presently described.

The Hypobromite Method of Hüfner (Knop).—This method is based on the fact that sodium hypobromite in alkaline solution decomposes urea into nitrogen, carbon dioxide and water, according to the following formula:

$$\underset{\text{Urea.}}{CO(NH_2)_2} + \underset{\text{Sodium hypobromite.}}{3NaOBr} = 3NaBr + CO_2 + 2H_2O + 2N$$

The CO_2 that develops is absorbed by the soda lye while the N passes through the lye and is measured; from the volume of N liberated the amount of urea can be calculated. This method is inaccurate in two respects: (1) the amount of N liberated is never quite as large as the theoretical amount; (2) hypobromite of soda also liberates N from other nitrogenous constituents of the urine than urea. Notwithstanding these two sources of error, the method is sufficiently accurate to merit general employment.

Hüfner's Apparatus (Fig. 4856) consists of three parts, viz.: (1) a cylindrical vessel, *A*, connected by a glass cock, *B*, with a smaller cylindrical receptacle, *C*; (2) a dish, *D*, that is connected with the upper end of *A* by a rubber cork (as shown in the figure), so that the open upper extremity of *A* protrudes into *D*; (3) an eudiometer tube, *E*, that should be from 30 to 40 cm. long, 2 cm. wide, and should be graduated for 0.2 cm. The eudiometer tube can be held in an inverted position over the upper end of *A* by a clamp, *F*, attached to a stand, *G*.

The Hypobromite Solution.—Seventy cubic centimetres of thirty-per-cent. sodium hydrate solution are diluted with 180 cc. of distilled water; the solution is then mixed with 5 c.c. of bromine. The bromine dissolves in the lye, and sodium bromide and sodium hypobromite are formed according to the formula:

$$3NaOH + Br_2 = NaBr + NaBrO + H_2O$$

The hypobromite solution should be kept in a cool, dark place in a tightly stoppered bottle, and should be made fresh every few days, as otherwise the sodium hypobromite is apt to decompose into sodium bromide.

Method of Performing the Determination.—The urea content of the urine is first estimated from the specific gravity (see above) and the urine diluted so as not to contain more than one per cent. of urea. Of this diluted urine exactly 5 c.c. are allowed to flow from a long pipette into the small receptacle, *C*, care being taken not to spill any of the urine in *A*, the pipette is carefully rinsed with water, and these washings also allowed to flow into *C*, until the latter receptacle is filled to the cock, *B*. The cock is now closed. *A* is now filled to overflowing with hypobromite solution; the dish, *D*, and the eudiometer tube, *E*, are then filled with concentrated NaCl solution and the latter inverted in the former and held over the open upper end of *A* by the clamp, *F*. The cock, *B*, is now opened. As the hypobromite solution is heavier than the urine, it runs down through *B* into *C* and at once causes an active development of gas (CO_2 and N). The CO_2 is absorbed in the sodium hydrate solution contained in *A*, while the N rises up through *A* and collects in the tube *E*. The reaction is over in about twenty minutes. The eudiometer is then transferred to a deep vessel filled with water of room temperature and allowed to stand there for fifteen minutes; the reading is finally made in such a way that the levels of the fluid in the vessel and in the tube are made equal. At the same time the temperature of the water and the barometric pressure are noted.

The volume of gas must now be reduced to 0° C., 760 mm. and absolute dryness; this is done according to the formula:

$$V' = \frac{V\,(B - W)}{760\,(1 + 0.0036\,t)\,354.3}$$

V′ = Volumes of nitrogen (corrected).

V = Volume indicated on eudiometer.
B = Barometric pressure.
W* = Tension of water vapors at temperature t.
t = Temperature of water.
0.0036 = Coefficient of expansion of gases for 1° C.

As urea only yields 354.3 c.c. of N instead of the theoretic amount of 372.7 c.c., when it is disassociated with hypobromite solution, V′ must be divided by 354.3 in order to obtain the correct amount of N developed, for

$$354.3 : 1 = V' : x$$
$$x = \frac{V'}{354.3}$$

If 5 c.c. of urine were employed for the determination, V′ multiplied by 20 gives the percentage of urea.

A simpler apparatus, called a ureometer, has been constructed on a similar principle as Hüfner's apparatus, but the results obtained with this modified apparatus are altogether inaccurate and essentially valueless. These ureometers are unfortunately in common use.

Method of Mörner and Sjöquist.—The method is based on the following principle: If the urine is treated with barium chloride and barium hydrate in certain proportions, all nitrogenous constituents, with the exception of urea, are precipitated from the solution by a mixture of ether alcohol. In the filtrate the N is determined after Kjeldahl and the urea calculated from this amount of N.

Execution: Five cubic centimetres of urine (if necessary after removal of any albumin that it may contain) are treated in a small flask with 5 c.c. of baryta mixture (saturated solution of barium chloride plus five per cent. barium hydrate) and 100 c.c. of a mixture of one volume of ether and two volumes of alcohol (97 per cent.). The flask is allowed to stand for twenty-four hours; the precipitate is then filtered off, repeatedly washed with ether-alcohol; the filtrate treated with magnesia usta in order to drive off any ammonia that may be in solution, and the ether-alcohol driven off at a temperature of 55° C. The temperature should never be allowed to rise above 60° C. When the volume of the filtrate is reduced to 10 or 15 c.c, the N determination is made with a measured proportion of the liquid. The urea is calculated from the N by multiplying the latter with $\frac{15}{7}$, or 2.143.

Purin Bodies, Uric Acid and Its Congeners.—Uric Acid. Uric acid is a normal constituent of the urine. A healthy adult excretes from 0.2 to 1.25 gm. of uric acid in the twenty-four hours to from 30 to 35 gm. of urea, *i.e.*, about one to two per cent. of the urinary N appears in the urine in the form of uric acid.

The excretion of uric acid is subject to many fluctuations in health and in disease. The chief and probably the only source of uric acid are the nucleins of cell nuclei, and as these nucleins may be derived either from the food or from the cells of the tissues of the organism, the excretion of uric acid is determined on the one hand by the amount of nuclein-containing food that is ingested and on the other hand by the catabolism of the proper tissues of the body. The old view that uric acid is an oxidation product of *any* form of albumin has been shown to be wrong. The administration of very large quantities of albuminous pabulum *containing no nucleins* is not followed by an increase in the uric-acid excretion. The administration, however, of nuclein or of nuclein-containing food is always followed by increased excretion of uric acid. On the other hand, a subject fed for a long time on a diet containing no nucleins, or a subject after a prolonged period of fasting, still excretes appreciable quantities of uric acid; in the former case the increase of uric acid was derived from the food nucleins ("exogenous uric acid"); in the latter case the excreted uric acid was derived from the tissue nucleins ("endogenous uric acid"). Whereas the former factor is constant and independent of the individual in the sense, namely, that a definite quantity of food nuclein leads to the excretion of a definite and calculable quantity of uric acid, the latter factor is inconstant, varies in different individuals, and cannot be calculated in advance. It varies from 0.1 to 0.2 gm. a day in normal adults.

All attempts to find "normal" values or to determine the significance of fluctuations in the uric-acid excretion are altogether fictitious as long as the purin bodies of the food are not included in the calculation. The diversity of opinions among different authors in regard to the effect of albuminous food on the uric-acid excretion can be explained as follows: Those who argue that an albuminous diet increases the urinary uric acid experimented with a diet containing much meat (and consequently xanthin bases, nucleins, etc.), whereas those who claim that a proteid diet exercises no effect on the uric-acid excretion worked with vegetable albumins, milk, eggs, etc., all articles that contain little purin.

Pathologically the excretion of uric acid is increased in febrile disorders and in other diseases in which there is loss of tissue albumin; here the uric-acid increase usually proceeds *pari passu* with the urea increase. In leukæmia the uric acid is often absolutely increased; here the catabolism of many leucocytes must be made responsible. In gout and the uric-acid diathesis (goutiness) there is much divergence of opinion. The consensus of newer writers who looked to a constant diet of known nuclein content during their investigations seems to be that the uric-acid excretion is somewhat increased *during* the attack, and that immediately *preceding* the attack the uric-acid excretion is slightly decreased. In the intervals between the attacks the uric-acid excretion in gouty subjects differs in no way from that in normal subjects.

Qualitative Tests for Uric Acid.—See under urinary sediments (below).

Quantitative Estimation.—Of the many methods for determining the uric acid in the urine the method of Ludwig-Salkowski is the best, the most accurate, and the most rapid.

The method of Heintz was in universal use for many years. It is so simple that even to-day many clinicians employ it. While the results of this method are very inaccurate in the sense, namely, that far too little uric acid is indicated, it nevertheless has a place in the clinical laboratory for determinations of a *relative* increase or decrease of uric acid in the same individual. The method is carried out as follows: Two hundred cubic centimetres of urine are treated with 10 c.c. of concentrated hydrochloric acid; the mixture is allowed to stand for forty-eight hours and the crystalline deposit of uric acid filtered off; the crystals are washed with alcohol and ether and dried at 110° C.

The Method of Ludwig-Salkowski.—Uric acid is precipitated from urine that has been treated with an ammoniacal magnesia solution by ammoniacal silver nitrate solution. The resulting precipitate consists of silver-magnesium urate. (The chlorides remain in solution.) If this precipitate is treated with alkali sulphide, the silver is precipitated as silver sulphide, whereas the urate goes into solution as alkali-urate. From the latter solution uric acid crystallizes after acidulation and evaporation to a small volume. The crystals are gathered on a filter, washed and weighed as above. The addition of ammoniacal magnesia solution causes precipitation of triple phosphate, and this mingling with the gelatinous urate precipitate renders the latter more flocculent and easier to wash, etc.

Execution: Three solutions are required, viz.:

1. An ammoniacal silver solution. Twenty-six grams of silver nitrate are dissolved in water and so much ammonia added until the brown precipitate that forms is redissolved in the excess of ammonia. The solution is then filled up to 1,000 c.c. with water and kept in a dark place.

2. Magnesia mixture. One hundred grains of crystal-

*The values for W at ordinary temperatures are the following:

10° C. = 9,126	16° C. = 13,519	22° C. = 19,675
11° C. = 9,751	17° C. = 14,009	23° C. = 20,909
12° C. = 10,421	18° C. = 15,351	24° C. = 22,211
13° C. = 11,130	19° C. = 16,345	25° C. = 23,582
14° C. = 11,882	20° C. = 17,396	
15° C. = 12,667	21° C. = 18,505	

lized magnesium chloride are dissolved in water and a large excess of ammonia added. This leads to the precipitation of magnesium hydroxide. A solution of ammonium chloride that is saturated in the cold is now added in such quantity that the precipitate of magnesium hydroxide is redissolved. The moderately clear solution is filled up to 1,000 c.c.

3. Solution of sodium (or potassium) monosulphide. Ten grams of NaOH or 15 gm. of KOH are dissolved in 1,000 c.c. of water. One-half of this solution is saturated with hydrogen disulphide gas and then added to the other half. The KOH or NaOH employed for this preparation must be free from nitrate or nitrite, for this solution is added to the urate solution before it is acidulated; on acidulation any nitric or nitrous acid would be liberated and these acids decompose uric acid.

The concentration of these reagents is arranged so that 10 c.c. of each suffice for 100 c.c. of urine. It has been established that this proportion precipitates all the phosphates and urates, and, on the other hand, redissolves all the uric acid from the precipitates that form even if the urine contains a very large proportion of uric acid.

One hundred cubic centimetres of urine are poured into a beaker. Ten cubic centimetres of solution 1 and 10 c.c. of solution 2 are mixed in another vessel and treated with so much ammonia that the precipitate of silver chloride that forms is redissolved; sometimes a flocculent precipitate of magnesium hydroxide forms, but this does not interfere with the reaction. The reagent is slowly poured into the urine. A gelatinous precipitate forms that is allowed to settle partially and is then filtered off and washed with very weak ammonia water until the washings are free from silver and from chlorides. The precipitate is then brought from the filter into a beaker, either by perforating the filter and squirting the precipitate through, or, if a suction pump is used, by transferring the bulk mechanically with a glass rod and then rinsing the balance into the beaker with the stream from a wash bottle. Ten cubic centimetres of solution 3 are now mixed with 10 c.c. of water and heated to boiling. The filter is rinsed with this solution and the whole added to the suspension of the precipitate; the mixture is then heated to boiling over a small flame, the deposit of silver sulphide filtered off, frequently washed with hot water, washings and filtrate acidulated with dilute HCl (5 c.c. of HCl [1.12 specific gravity] diluted one-fourth are sufficient), and evaporated to about 20 c.c. On cooling, the uric acid crystallizes out. Some writers claim that all the uric acid is deposited after an hour; it is better to allow the solution to stand for at least six hours. The uric acid is filtered off, washed with alcohol and ether and weighed. For every 10 c.c. of the mother liquor and washings 0.00005 gm. of uric acid is added to the result, for this small amount of uric acid is soluble in 10 c.c. of water and consequently remains in solution.

Salkowski has modified this method slightly. Instead of decomposing the silver urate precipitate with alkali sulphide, he passes a stream of hydrogen sulphide gas through the suspension of silver urate in acidulated water; the silver salt is decomposed in the same way, the sulphide of silver being thrown down, while the uric acid passes into solution; the mixture is heated to boiling, filtered, and the filtrate treated as above. This modification has no advantages over the original Ludwig method—in fact, requires more care and more paraphernalia.

If sugar or albumin is present in the urine, they must be removed before the uric-acid determination is made. Ludwig advises removing the albumin as follows: The urine is treated with concentrated sodium chloride solution (10 to 15 c.c. to every 100 c.c. of urine), acidulated with acetic acid and boiled. The albumins coagulate and can be removed by filtration; the filtrate must be filled up to the original volume of the urine.

If the urine contains uric-acid crystals, these must be dissolved by warming before the urine is measured off. The precipitation of urates may often be prevented by adding a pinch of sodium bicarbonate to the urine immediately after it is voided.

Of other methods for determining uric acid only the underlying principles will be given in this place; for details I refer to the text-books.

Method of Hopkins. Thirty grams of ammonium chloride are dissolved in 100 c.c. of the cold urine. This leads to the precipitation of ammonium urate; the latter is filtered off and washed, then dissolved in boiling water and acidulated with HCl; after concentration of the solution and cooling the uric acid crystallizes out and can be weighed as above.

Some authors prefer to determine the amount of uric acid by *titration*, either acid metrically or with permanganate of potassium, or as silver magnesium salts (see Huppert, "Harnanalyse," 1898, p. 814).

Method of Folin. The uric acid is precipitated with the following reagent: 500 gm. of ammonium sulphate, 5 gm. of uranium acetate, 60 c.c. of a ten-per-cent. solution of acetic acid dissolved in 650 c.c. of water; 75 c.c. of this reagent are added to 300 c.c. of urine. Soon a mucoid body separates from the urine that is removed by filtration; the filtrate is then treated with concentrated ammonia; this leads to the precipitation of ammonium urate. The latter is washed into a beaker, treated with concentrated sulphuric acid, and titrated at once with one-twentieth decinormal solution of potassium permanganate. Each cubic centimetre of the reagent corresponds to 0.00375 gm. of uric acid. As ammonium urate is slightly soluble a correction of 0.003 gm. must be made for every 100 c.c. of urine.

Purin Bases. (Synonyms: Xanthin Bases, Nuclein Bases, Alloxuric Bases). To this group belong many substances, of which the following have been found in human urine: Xanthin, heteroxanthin, paraxanthin, guanin, hypoxanthin, adenin, episarcin, carnin, epiguanin, and "the unknown basis of Krüger and Wulff." The most important of these bodies is xanthin. The quantity of purin bases occurring in the urine is very small. On a mixed diet the urine voided in twenty-four hours contains 87 gm., on an animal diet 44 mgm., and on a vegetable diet (peas and cabbage) 72 mgm., or (apples and carrots) 111 mgm. If the uric-acid nitrogen voided on these different varieties of diet is calculated at 100, the nitrogen of the purin bases (figured for xanthin) equals 18.1, 7.6, 18.1, and 35.8. The amount of xanthin bases excreted, therefore, equals only a fraction of the uric acid excreted. The daily normal excretion equals from 0.015 to 0.06 gm. Of this amount 0.015 to 0.03 is xanthin; hypoxanthin and guanin are next in importance, then come paraxanthin and heteroxanthin. The other members of the group are of rare occurrence and of very subordinate importance.

The purin bases are derived from nuclein; they may consequently, like uric acid, be derived from the food nucleins or from the tissue nucleins. Their relation to uric acid is as follows: If nuclein or nuclein-containing organs (spleen pulp) are allowed to stand at body temperature with free access of oxygen, uric acid is formed; if oxygen is withheld, the purin bases are formed instead. It is possible to convert purin bases into uric acid by the aid of organ extracts, for the latter apparently contain an "oxydase" that can produce this oxidation. Pathogenetically the purin bases probably play a rôle in the uric-acid diathesis, for, as I have shown, they can cause certain renal lesions that are identical with the lesions of granular nephritis in gout, and also certain cardiovascular changes that are similar to those seen in nephritis, arteriosclerosis, etc. Much has been written in regard to the relation existing between the elimination of uric acid and of the xanthin bases. It was believed for a time that a decrease of the uric-acid excretion was compensated by a corresponding increase in the purin-base excretion in gout and goutiness. These findings, including my own, have since, however, been shown to be uncertain, owing to the fact that the method for the determination of purin bases (Krüger and Wulff) was deficient. Both uric acid and purin bases may, of course, also be increased or decreased together. In leukæmia the greatest amount of purin bases (and of uric acid) is excreted. Tea and coffee, owing to the theobromine (dimethyl xanthin) and caffeine (trimethyl xanthin) they incorporate lead to the excretion of methylated xanthin and heteroxanthin. Xanthin sometimes forms a crystalline sediment in the urine. This will be described under urinary sediments.

For the present the quantitative determination of the purin-base excretion is of subordinate clinical importance; the method, moreover, is complicated and requires more skill and more apparatus than are usually at the disposal of the clinician and practitioner. The following *method of Salkowski* gives accurate results. The method is based on the precipitation of uric acid and purin bases together as silver salts and the subsequent separation of the latter from this precipitate.

Execution: A measured quantity of urine is treated with ammoniacal magnesia mixture and ammoniacal silver solution, as described above (Ludwig-Salkowski method) under "uric acid." The uric acid is removed as in the Ludwig method, and the final filtrate, which contains the purin bases, together with the washings, treated with ammoniacal silver solution. This reprecipitates the purin bases. The precipitate is collected on a filter, washed, dried, incinerated. The ash is dissolved in dilute nitric acid and the silver estimated in this solution by titration. The best method of titration is with potassium sulphocyanide, using ammonio-ferric alum as an indicator. In a compound of the silver salts of xanthin, hypoxanthin, etc., one atom of silver represents 0.277 gm. of nitrogen, or 0.7381 gm. of the bases. Consequently 1 c.c. of the sulphocyanide solution will correspond to 0.002 gm. of nitrogen, or 0.00542 gm. of the bases. The sulphocyanide solution is made up as follows: The solution should contain 12.9849 gm. to a litre. The salt is very hygroscopic, so that it cannot be weighed accurately. Therefore a concentrated solution is made and standardized against a silver solution containing 29.042 of silver nitrate to the litre, using ammonio-ferric alum as an indicator.

The separation and identification of the different members of the group of purin bases is possible, but it requires very much time and considerable chemical skill. As this separation is of no clinical value, but merely of physiological interest, it will not be described in this article.

Nucleinic Acid.—This body occurs in small quantities in the urine either in combination with a proteid molecule (see nucleo-albumin), or occasionally free. Nucleinic acids are a combination of purin bases, phosphoric acid, and of a nitrogen-free molecule (possibly pentose). They contain as much as 9.5 per cent. of phosphorus; nucleo-albumins contain about 1.5 per cent. of phosphorus. Nucleinic acid is precipitated from the urine together with chondroitin sulphuric and taurocholic acids (icterus) if the greater portion of the inorganic salts are removed from the urine; the latter are therefore removed by dialysis, the liquid treated with dilute acetic acid, and the precipitate removed by filtration; the filtrate of urine containing no albumin is now treated with a small quantity of blood serum (1.5 c.c. to a litre) and allowed to stand. The precipitate contains the nucleinic acid, together with chondroitin sulphuric and taurocholic acids (icterus). The presence of nucleinic acid is finally determined by determining the presence of phosphorus and of purin bases.

Allantoin.—This body is occasionally found in the urine of new-born children (during the first week of life), in the urine of pregnant women, and occasionally in the urine of men. It is a normal constituent of cat's, dog's, and rabbit's urine. It appears to be an intermediary product in the oxidation of uric acid; but it does not occur in the urine of man after excessive feeding with nuclein-containing food (Loewi). Its clinical significance is so small that methods for detecting and estimating allantoin will not be given.

Kreatin (Kreatinin).—Kreatin has occasionally been found in the urine of man. It is rapidly converted into kreatinin, however, and the latter substance is constantly found in the urine in quantities varying from 0.6 to 1.3 gm. in the twenty-four hours' quantity. It is derived from the disassimilation of muscle tissue either of the food or of the organism proper. In the muscles of the body kreatin is always formed first, and this is secondarily converted into kreatinin in its further passage through the blood and tissues. The excretion of kreatinin is chiefly dependent on the diet—much meat, much kreatinin. But even if all muscle meat is withheld, or if the meat is first leached out with water (Rubner) a certain amount of kreatinin continues to be excreted. Physical exercise seems to increase the kreatinin excretion.

Pathologically kreatinin has been found increased in febrile disorders of various kinds, and in diabetes. It has been found decreased in chronic nephritis, in diabetes insipidus, and in the period of convalescence from acute diseases, also in anæmia, chlorosis, tuberculosis. The clinical significance of a plus or minus in the kreatinin excretion is so far not understood. More casuistic data must be collected before this sign can be utilized in the interpretation of morbid states.

Tests.—Qualitative. Weyl's Test.—A small quantity of the filtered urine is treated with a few drops of a ten-per-cent. solution of sodium hydrate and one drop of a ten-per-cent. solution of sodium nitroprusside. If the urine contains kreatinin, it turns ruby red and then yellow. If the urine, after it has turned yellow, is now acidulated with acetic acid and heated, it turns green, then blue, and finally precipitates a blue sediment. This reaction does not occur in the cold. Instead of acetic acid certain other organic acids can be employed.

Kreatin does not give this test, neither do allantoin, xanthin, leucin, tyrosin, and other bodies that are genetically related to kreatinin. The only other urinary constitutent (with the exception of certain rare hydantoins) that gives a similar color test is *acetone;* on acidulation with acetic acid the liquid, however, turns red with acetone, whereas with kreatinin it turns blue. It is easy to drive off any acetone that may be present by first boiling the urine for a few minutes.

Test of Jaffé.—The urine is treated with a few drops of dilute sodium hydrate and a few drops of picric acid; the liquid at once turns red, even in the cold. This reaction can still be obtained in the presence of 1 part of kreatinin to 5,000 parts of water. No other known constituent of the urine gives this test.

Quantitative Determination.—Kreatinin forms a double compound with chloride of zinc—that is essentially insoluble in alcohol (1 to 9,217). This property is utilized for the quantitative determination.

Execution: Two hundred to 300 c.c. of urine are rendered alkaline with lime water and treated with a solution of calcium chloride so long as a precipitate continues to form. The mixture is allowed to stand for one or two hours and filtered. The filtrate is slightly acidulated with sulphuric acid and evaporated to a syrupy consistence in the water-bath. While still warm it is treated with 40 to 50 c.c. of alcohol of 95 per cent. The mass is thoroughly mixed washed into a beaker with a small quantity of 95-per-cent. alcohol and allowed to stand in a cool place for from six to eight hours. The precipitate is filtered off and washed and the filtrate and alcoholic washings evaporated back to about 50 c.c. This solution is now treated with 0.5 c.c. of an alcoholic chloride-of-zinc solution and allowed to stand for two or three days. The crystals of kreatinin-zinc chloride are then gathered on a weighed filter, repeatedly washed with alcohol until the filtrate shows no chlorine reaction, dried and weighed. One gram of kreatinin-zinc chloride represents 0.6242 gm. of kreatinin. In order, therefore, to determine the amount of kreatinin present in the quantity of urine employed for the determination, the figure obtained from weighing the zinc compound must be multiplied by 0.6242.

The reagent employed for precipitating the kreatinin-zinc compound (*i.e.*, the alcoholic solution of zinc chloride) is made by treating a saturated watery solution of zinc

chloride with alcohol until the mixture has a specific gravity of 1.200.

Oxaluric and Oxalic Acids.—Oxaluric acid occurs only in insignificant traces in normal urine, usually in the form of the ammonium salt. Oxalic acid is always present in normal urine. It usually occurs as the calcium salt, which is held in solution by diacid sodium phosphate. It precipitates with facility on standing and then forms characteristic crystals (see urinary sediments). Whereas oxalic acid $\left|\begin{matrix}\text{COOH}\\ \text{COOH}\end{matrix}\right.$ contains no nitrogen, it may nevertheless be discussed in this place in connection with oxaluric acid, as both these substances are chemically and, probably, genetically closely related. Oxaluric acid readily splits up into oxalic acid and urea according to the formula:

$$\underset{\text{Oxaluric acid.}}{\left|\begin{matrix}\text{CO.NH}\\ \text{COOH.NH}_2\end{matrix}\right.} > \underset{\text{Water.}}{\text{CO} + \text{H}_2\text{O}} = \underset{\text{Oxalic acid.}}{\left|\begin{matrix}\text{COOH}\\ \text{COOH}\end{matrix}\right.} + \underset{\text{Urea.}}{\text{CO}\begin{matrix}\diagup\text{NH}_2\\ \diagdown\text{NH}_2\end{matrix}}$$

Both may be derived from uric acid, although oxalic acid may also be derived from other sources. It has recently been shown to be an intermediary produced in the degradation of dextrose. It may further be derived from certain articles of food, as asparagus, apples, grapes, and spinach. It is possible that a certain amount of the urinary oxalic acid is derived from this source.

The normal oxalic-acid excretion is about 0.02 gm. in twenty-four hours. Oxalic acid is occasionally found increased in diabetes. Some clinicians speak of vicarious oxaluria in this disease, for it seems that the sugar excretion decreases occasionally as the oxalic-acid excretion increases. If oxalic acid could ultimately be shown to be an oxidation or a splitting product of blood-sugar (see above), this peculiar relation would be easily explained. Oxaluria has finally been vested with the dignity of an independent clinical entity (oxalic-acid diathesis, idiopathic oxaluria). The syndrome of this condition is rather vague: the chief manifestations being a variety of subjective symptoms, emaciation, and a great increase in the excretion of oxalic acid (as much as 0.5 gm. in 1,000 c.c. of urine). No pathologic organic lesions are discoverable.

Estimation of Oxalic Acid. Qualitatively crystals of oxalic acid can be recognized by their solubility in HCl and their insolubility in acetic acid (see also "urinary sediments"). The quantity of oxalic acid is determined as follows:

Method of Neubauer.—The total quantity of the urine excreted in twenty-four hours is treated with calcium chloride and ammonia, then slightly acidulated with acetic acid and allowed to stand until the sediment settles at the bottom of the vessel. It is advised to add a few drops of an alcoholic thymol solution in order to prevent the excessive development of micro-organisms, for the latter may render subsequent clearing of the urine by filtration difficult. The precipitate is filtered off and placed into dilute hydrochloric acid together with the filter, the liquid heated a little, filtered, and the residue washed with water until the filtrate is no longer acid. The filtrate and washings are then evaporated to a small volume; while still hot this liquid is oversaturated with ammonia and colored with a few drops of litmus tincture as an indicator of the reaction. The solution is allowed to stand for twenty-four hours and the crystals of calcium oxalate gathered on a weighed filter; the weight of the filter ash must be known. The precipitate is washed free from chlorides, dried, incinerated in a platinum crucible and glowed to constant weight. This converts the oxalate of calcium into the oxide of calcium. Fifty-six parts of calcium oxide correspond to ninety parts of oxalic acid. The weight of the calcium oxide must, therefore, be multiplied by $\frac{90}{56} = 1.6071$, in order to give the amount of oxalic acid present in the total quantity of urine used for the estimation.

There are several sources of error in this method that render the results not quite accurate in the sense that too little oxalic acid is indicated. In view of the small quantities of oxalic acid excreted in the urine these small errors become relatively large. Other methods (Baldwin [Dunlop], Salkowski) in the writer's experience are fully as complicated as the old method of Neubauer, and no more accurate. In view of the slight clinical significance of small fluctuations in the oxalic-acid excretion and the small need of performing quantitative estimations these other methods will not be described in this article.

Carbohydrates.—Normal urine always contains small quantities of carbohydrates, notably the following three: animal gum, dextrose, and isomaltose. Chemically related to these are the conjugate glycuronates, and certain glucosides (chondroitin-sulphuric acid, nucleinic acid, and "mucin"). Occasionally pentose is also found in normal urine. Under pathological conditions the urine may contain levuloses (laiose) and certain polysaccharides, viz., erythrodextrin and glycogen.

Carbohydrates in General.—Certain reactions are common to all carbohydrates occurring in normal urine. They may consequently be utilized for the detection of these bodies.

(1) *The Benzol-ester Reaction* (*Baumann*). If the urine is shaken with benzoyl chloride and a quantity of sodium hydrate added sufficient to decompose the latter, a precipitate of benzoyl compounds is formed that incorporates dextrose, isomaltose, and animal gum.

(2) *The Furfurol Reaction.* Carbohydrates are decomposed by sulphuric acid, and one of the products of this decomposition is furfurol. The latter, even in very minute quantities, gives definite color reactions with certain reagents, viz., *a*-naphthol, thymol, xylidin, and resorcin. In addition to glucose, isomaltose, animal gum, and pentoses, albumen also gives this reaction with *a*-naphthol. In performing the test albumen should therefore be removed first.

(3) *The Reduction of Metal Oxides.* Normal urine reduces cupric and mercuric oxide in alkaline solution, and can also convert orthonitrophenyl propionic acid in alkaline solution into indigo blue. This method can hardly be utilized, however, to determine the presence of carbohydrates in normal urine. It is important merely to remember that normal urine, owing to the presence of certain reducing carbohydrate bodies possesses slight reducing powers, and that this factor must be included in determining the cupric-reducing powers of pathological urines.

Carbohydrates are commonly divided into monosaccharides, disaccharides, and polysaccharides. The di- and polysaccharides may be considered condensation products of monosaccharides with loss of water. Inversely di- and polysaccharides can be split by hydrolytic decomposition (addition of water) into the original component monosaccharide molecules.

Monosaccharides.—We distinguish among the monosaccharides trioses, tetroses, pentoses, hexoses, heptoses, etc., according to the number of carbon atoms the molecules contain. Of these different sugars the pentoses and the hexoses (*i.e.*, the sugars containing five and six combinations) are alone of physiological and pathological interest.

Pentoses. See article on *Pentosuria.*

Hexoses. Glucose (grape sugar, glycose, dextrose) $C_6H_{12}O_6$. As already stated, normal urine always contains traces of glucose. After the abundant ingestion of glucose, of disaccharides and polysaccharides, the excretion of monosaccharides (dextrose or levulose) in the urine may increase. This condition is called *alimentary glycosuria* (*e saccharo*).

Glycosuria (synonyms glycuria, melituria), *i.e.*, the excretion of dextrose in the urine, may occur in a great variety of conditions.

Certain drugs, as *uranium salts, corrosive sublimate, phosphoric acid* (intravenously), lead to the excretion of dextrose; certain other drugs, as *chloralamide, chloral, nitrobenzol,* and *nitrotoluol, orthonitro propionic acid,* lead to the excretion of reducing substances that are frequently taken for sugar. On careful examination it will be found, however, that these reducing substances rotate the plane of polarized light to the left, and are therefore not dextrose, and that they do not ferment with yeast, and are therefore not levulose. As a matter of fact, the bulk of these reducing substances consists of glycuronic acid compounds that these drugs form in their passage through the body. It is interesting to note, however, that in addition to these conjugate glycuronates, dextrose may appear in the urine at the same time.

Certain drugs that lead to cellular *asphyxia,* as *carbon monoxide, amyl nitrite, curare, methyldelphinin, strychnine, morphine, chloroform, ether,* and other drugs that produce asphyxia by narcosis, may all produce glycosuria.

Phloridzin produces sugar excretion in a different way than any of the other poisons enumerated. Whereas in all the glycosurias mentioned the excretion of sugar is preceded by the accumulation of abnormally large quantities of sugar in the blood, *scil.* hyperglycæmia, in phloridzin glycosuria the blood-sugar is not increased above normal. The exact pathogenesis of phloridzin glycosuria is still a subject of controversy. Phloridzin is a glucoside made from the bark of the roots of apple and cherry trees. On boiling with acids it decomposes into phloretin and phlorose, the latter being a monosaccharide that is closely related to glycose. One gram of phloridzin per kilogram of body weight administered to dogs by the mouth leads to the excretion of as much as eighteen per cent. of dextrose in the urine; the excretion of sugar may continue for two or three days after the administration of a single dose. In man von Mering caused the daily excretion of about 100 gm. of dextrose for thirty consecutive days by injecting 2 gm. of phloridzin every day. As soon as the exhibition of phloridzin was stopped, the excretion of dextrose also ceased.

Phloridzin glycosuria is without doubt due to some *renal* process. This is clearly shown by the absence of hyperglycæmia, even though the ureters are ligated or the kidneys extirpated. In fact, phloridzin injected into one of the renal arteries at first leads to the excretion of dextrose only from this one kidney; later the other kidney also begins to excrete sugar.

Two possible explanations can be proffered in the light of our present knowledge, viz.: either phloretin damages the renal epithelium in the sense that the latter becomes abnormally permeable for dextrose, or phloridzin is disintegrated into its two components in the kidney—the sugar component being excreted, the phloretin returning into the circulation, recombining with sugar to form phloridzin, and finally again undergoing decomposition in the kidneys. If the latter assumption is correct, the process of sugar excretion would be continued until finally the phloretin itself would be completely eliminated. A strong argument in favor of this theory is the observation that phloretin itself can produce glycosuria. In view of the fact finally that changes in the renal epithelia have never been observed after the administration of phloridzin, the latter pathogenesis is the one now almost universally accepted.

Glycosuria also appears after injuries to the *liver,* and in many chronic diseases of this organ. It may appear in the course of a great variety of *nervous disorders* (viz., apoplexy, brain tumor, encephalomalacia, cerebrospinal meningitis, dementia paralytica, multiple sclerosis, tabes dorsalis, traumatic neurosis, various functional neuroses, and in certain psychoses). In many of these disorders the sugar does not spontaneously appear in the urine, but only after the administration of carbohydrate pabulum in quantities so small that it would not lead to sugar-excretion in a normal subject. Here, then, the power of *assimilating* carbohydrate is merely reduced, hence the glycosuria.

Glycosuria may accompany *Basedow's disease, myxœdema, acromegaly,* and *Addison's disease;* sugar appears in the urine after the *injection of thyroid and suprarenal extracts.*

In *gout, obesity,* and *arteriosclerosis,* in certain *diseases of the pancreas,* in *syphilis,* in *cholera, malaria, carbuncle,* and other infections, sugar may appear in the urine.

Finally, glycosuria is the most typical symptom of *diabetes mellitus* and of *experimental diabetes* occurring in depancreatized animals.

Tests for Glucose. No simple test exists for the quantitative detection of glucose to the exclusion of all other sugars that may appear in the urine. From a clinical standpoint this differentiation is fortunately not very important; hence I will not describe the complicated methods we possess for isolating and identifying dextrose in a mixture of sugars and other carbohydrates and carbohydrate derivatives. Of the many urinary sugar tests we possess the following are the most practical and the most simple:

Fehling's Test (modification of Trommer's test).—Trommer's test was carried out as follows: The urine was rendered alkaline with potassium hydrate solution; a strong solution of copper sulphate was then added drop by drop until a portion of the cupric hydrate remained undissolved; the mixture was then heated. If appreciable quantities of sugar were present, yellow or red cupric oxide was precipitated even before the boiling point was reached, while at the same time the liquid became somewhat decolorized. This test is quite sensitive. Trommer was able to detect the presence of 0.001 per cent. of sugar. Unfortunately a large number of substances other than sugar can produce the same reaction—for instance, uric acid, kreatinin, allantoin, nucleoalbumin, bile pigments, conjugate glycuronates, etc. If the reduction occurs before the mixture is boiled, this reaction is always due to sugar. It only occurs, however, if the percentage of sugar is large.

Fehling modified this test by substituting a mixture of potassium-sodium tartrate (Rochelle or Seignette salts) and caustic alkali for the simple caustic alkali solution. Fehling's alkaline solution is made up as follows: 173 gm. of Rochelle salt and 120 gm. of sodium hydrate are dissolved in 500 c.c. of water. For quantitative work (see below) it is best to first prepare a sodium hydrate solution of 1.34 specific gravity and to dissolve 100 c.c. of this lye with 173 gm. of Rochelle salt in the necessary amount of water. The copper-sulphate solution should contain 34.63 gm. of copper sulphate to 500 c.c. of water. The two solutions should be kept separately. Before performing the test equal parts of the solutions are mixed and diluted with five volumes of water. As sugar is the only important urinary ingredient that reduces an alkaline copper solution at a temperature below the boiling point (*i.e.*, at about 80°), the author prefers to perform the Fehling test as follows: Equal portions of the urine and of the reagent are poured into two test tubes and each tube heated to boiling; both tubes are then allowed to cool off a little and the reagent then poured into the urine. If sugar is present, the yellow or red oxide of copper will appear almost at once and all through the liquid. This method of performing the test, aside from being more positive for sugar, is much more delicate for small quantities of dextrose.

Nylander's Bismuth Test.—This test is based on the same principle as the Trommer-Fehling test—*i.e.*, the power of dextrose to reduce metallic salts. Here the reduction of subnitrate of bismuth is utilized instead of the reduction of sulphate of copper. The test has this advantage over the Fehling test—that uric acid, kreatinin, and homogentisinic acid do not reduce bismuth subnitrate. After rheum, senna, salol, antipyrine, quinine, the urine, however, always gives this test. The presence of uroerythrin or of hæmatoporphyrin may simulate a reduction. If the urine contains much ammonia salt, the test may fail even in the presence of dextrose.

Nylander's reagent consists of 4 gm. of sodium and potassium tartrate, 2 gm. of bismuth subnitrate, and 10 gm. of sodium hydrate dissolved in 90 c.c. of boiling water. The solution, after cooling, must be filtered and preserved in a dark bottle.

Ten parts of the urine are treated with one part of the reagent and boiled for several minutes (at least two to three minutes!). It is imperative to *boil for some time.* If dextrose is present, a gray or dark-brown precipitate

of bismuthous oxide or a black precipitate of metallic bismuth will form.

Fermentation Test.—Glucose is split into alcohol, carbon dioxide, and certain other products (*i.e.*, succinic acid, glycerin, etc.) by the action of yeast.

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} = \underset{\text{Alcohol.}}{2C_2H_6O} + \underset{\text{Carbon dioxide.}}{2CO_2}$$

According to this formula, which represents the chief reaction that occurs, a given quantity of sugar should yield 51.1 per cent. of alcohol and 48.9 per cent. of carbon dioxide. As a matter of fact, only 48.67 per cent. of alcohol and 46.54 per cent. of carbon dioxide are formed, the deficit being made up by 3.71 per cent. of glycerin and succinic acid and 0.94 per cent. of unknown substances. It will be seen, however, that the proportion between the two main products remains the same, viz., as 1.045:1 as called for by the equation. The temperature optimum for the alcoholic fermentation of sugar lies at 34° C. Below 15° C. the process is not completed, and above 45° C. it does not take place at all. Sugar solutions of from four to eight per cent. ferment more rapidly than more or less concentrated solutions. The proper proportion of CO_2 (46.54 per cent.) is only obtained if not more than one-half part of fresh doughy yeast is used for one part of sugar. More yeast leads to the development of more CO_2, owing to the auto-fermentation (development of buds) of the yeast that in itself leads to the generation of CO_2.

The test is performed as follows: A little compressed yeast is shaken with about 25 c.c. of urine and the mixture placed into a saccharometer (Einhorn's saccharometer). If glucose is present CO_2 will be developed at room temperature, or better at 34° C. The gas accumulates at the top of the tube. Before performing this test the yeast should be placed into a glucose solution in order to determine whether it is active. As normal urine frequently ferments a little, a control tube should be compared with the tube to be tested. If the percentage of sugar is very small, the CO_2 may be absorbed before it rises to the top of the tube. In case the urine gave a positive Fehling or Nylander test, but apparently does not ferment, it is well to repeat one of the reduction tests after the urine has been kept in the fermentation tube for twenty-four hours. If at the end of this time the urine no longer reduces copper or bismuth salts, then we may know that the original reduction was due to fermentable carbohydrate (glucose). If it still reduces Fehling's or Nylander's solution, then we may conclude that the original reduction was wholly or in part due to the presence in the urine of unfermentable carbohydrates.

The Phenylhydrazin Test.—This test was formerly considered quite pathognomonic for pathologic sugars in the urine. Since it has been shown, however, that normal urine, when treated with phenylhydrazin may occasionally precipitate crystals that resemble in appearance the typical glucosazon, the validity of this test seems impaired. The author, it is true, has never so far succeeded in obtaining such crystals from normal urine, but statements to this effect are made by competent authorities.

In addition to certain sugars (see below) the pentoses and glycuronic acids give osazons with phenylhydrazin. Acetone and diacetic acid give a hydrazin that is almost insoluble; oxalic acid gives a salt that is not very soluble; urea in concentrated urines gives a peculiar compound with phenylhydrazin. While acetone and oxalic acid will probably never be confounded with sugar, and while urea is probably never present in sufficient amounts in human urine to produce the formation of the phenylhydrazin compound, and while, finally, free glycuronic acid probably never occurs in the urine, nevertheless all these possible sources of error must be considered when interpreting the significance of a phenylhydrazin crystallization.

Our chief aid in differentiating the different osazons that are formed from various sugars is the melting point,* as this differs so considerably that the osazons can usually be distinguished by this means alone.

The test is performed as follows: 10 c.c. of urine are poured into a test tube and to it are added 0.5 to 0.75 gm. of phenylhydrazin, and not quite twice as much (1 to 1.5 gm.) of sodium acetate. With a little practice the amount of phenylhydrazin and the proportion of sodium acetate can be estimated with sufficient accuracy. The mixture is heated to boiling, and if all the salts do not dissolve readily, a little water is added. The tube is then placed into boiling water for from twenty to thirty minutes and then allowed to cool slowly. It is best to turn off the flame under the water-bath and to allow the tube to cool in the bath. At the expiration of half an hour the tube is placed into cold water. In this way better crystals are obtained. A number of rapid tests have been described; but I find the technique described above to be absolutely reliable, whereas I have on several occasions known the "five-minute tests" to fail; this applies particularly to urines containing only small amounts of sugar. As soon as the tube is placed into cold water, if not before, a yellow crystalline precipitate of phenyl-glucosazon appears. Sometimes this sediment appears amorphous on naked-eye inspection. On microscopic examination, however, it will be found to consist of fine needles that are arranged in bundles and sheaths. They are of a bright yellow color. The precipitate must present this microscopic appearance; flakes and plates of yellow material prove nothing in regard to the presence of sugar. In doubtful cases a melting-point determination of the crystals must be made. (For the technique see text-books on chemistry.)

This test can be performed even if the urine contains small quantities of albumin. Large quantities of albumin are preferably first removed by boiling and filtration. The test is very delicate and indicates the presence of 0.1 per cent. of sugar. The test requires a little practice, but after the technique is once mastered it constitutes one of the most satisfactory and reliable tests for sugar, particularly in combination with one of the reduction tests.

Polarimetric Examination.—For the qualitative recognition of glucose in the urine the polariscopic test is not very satisfactory because many other substances besides sugar can turn the plane of polarized light to the right or the left. The presence of glucose should, therefore, never be diagnosed from dextro-rotation alone. If a specimen of urine gives one of the above tests, and if in addition it shows marked dextro-rotation, this evidence may be considered highly corroborative of the presence of dextrose. Normal urine turns the plane of polarized light slightly to the left; albumen is also levorotary; biliary acids are dextrorotary. All these bodies can be precipitated from the urine by sugar of lead. This source of error can, therefore, be eliminated by treating the urine with sugar of lead and making a polarimetric examination of the filtrate; dextro-rotation may appear in the latter, whereas it was previously neutralized by the presence of levorotary substances in the urine. Other levorotary bodies that may mask the dextro-rotation produced by small quantities of glucose are levulose, conjugate glycuronates, and β-oxybutyric acid. If the urine is allowed to ferment and is examined with the polariscope before and after fermentation, the error introduced by the presence of these levorotary reducing substances may occasionally be detected and controlled (see Glycuronates below).

For a detailed description of the polarimeter and the technique of polarimetry I refer to special text-books on physics.

* *Melting Points of Various Osazons.*

Glucose	204–205° C.
Levulose	204–205° C.
Galactose	193° C.
Maltose	206° C.
Isomaltose	150–153° C.
Lactose	200° C.

There are many other qualitative tests for sugar, but their description lies beyond the scope of this article. The ones given are the best; they are sufficient for all clinical purposes.

Quantitative Estimation of Glucose.—By Titration. (*a*) According to Fehling.—In pure sugar solutions the sugar percentage can be determined by weighing the cuprous oxide precipitate. In the case of the urine this cannot be done because a portion of the oxide is held in solution by the ammonia that develops when urine is boiled with Fehling's alkaline solution. It is possible, however, to determine the volume of urine that is required to reduce the cuprous oxide salt in a definite volume of Fehling's solution. The correct volume of urine is determined by observing the exact point at which the blue Fehling's solution becomes discolored. In observing the color change one should not wait too long, but should examine as soon as the cuprous oxide begins to settle and leaves a narrow zone of clear fluid on top. If one waits too long, a bluish tinge may return owing to the oxidation of that moiety of the cuprous oxide that is held in solution by ammonia. In case the cuprous oxide does not settle rapidly the addition of some sodium hydrate hastens the precipitation.

The specific gravity of the urine having been determined in order approximately to estimate the amount of sugar it contains (see above), it should be diluted so far that it does not contain more than 0.5 per cent. It is good practice to always dilute diabetic urine five to ten times. The dilute urine is poured into a burette; 5 c.c. of the alkaline and 5 c.c. of the cupric solution (see above for Fehling's solution) are now mixed and diluted with 40 c.c. of water. The solution is heated to the boiling point and the urine allowed to flow into it slowly and carefully. A red or yellow precipitate of cupric or cuprous oxide forms and the blue color of the fluid disappears. The test should be repeated several times until the exact quantity of urine required to bring about the discoloration is determined to 0.1 c.c. A number of methods have been devised to determine the point of discoloration more accurately, but none of these methods have proven useful in the author's practice. For clinical purposes the above simple method is sufficiently accurate. In order to calculate the sugar percentage it must be remembered that each cubic centimetre of Fehling's solution corresponds to 5 mgm. of sugar; if 10 c.c. of Fehling's solution were reduced by a given quantity of urine, then this quantity of urine contained ten times 5 mgm., or 50 mgm., or 0.05 gm. of sugar. As the urine used for the test was diluted, the quantity of the original urine, that contains an equal amount of sugar, can be determined by dividing the cubic centimetres of diluted urine by the figure indicating how often the urine was diluted. For example, if 8.4 c.c. of diluted urine were required to reduce 10 c.c. of Fehling's solution, and if the urine was diluted six times, then the 0.05 gm. of sugar were contained in $\frac{8.4}{6}$ c.c. = 1.4 c.c. of the original urine; 100 c.c. of this urine, therefore, would contain 3.57 gm., or 3.57 per cent.

Method of Knapp.—This method does not require as much practice as Fehling's quantitative method. It is based on the fact that mercuric cyanide in alkaline solution is reduced by glucose to metallic mercury. In this test, too, however, there is an unavoidable source of error, for the urine always contains substances that can reduce the mercuric cyanide solution. For very accurate estimations the urine should, therefore, be fermented and its mercuric-reducing powers after fermentation determined. The figure obtained in this way should then be subtracted from the values for the total reducing powers; for ordinary clinical purposes this correction is superfluous.

For performing this test the urine should be freed from albumin by boiling with dilute acid and filtering. The urine should not contain more than one per cent. of sugar by a preliminary test or by estimation from the specific gravity; the sugar percentage should first be estimated, and, if the percentage is too high, the urine correspondingly diluted.

The reagent should contain 10 gm. of pure dry mercuric cyanide and 100 c.c. of sodium-hydrate solution of the specific gravity of 1.145 (or 13.3 gm. of sodium hydrate) to the litre. The reagent is conveniently made by dissolving 10.754 gm. of chemically pure mercuric chloride in a little water and adding so much cyanide of potassium that a drop of sodium-hydrate solution no longer produces a precipitate; to this mixture are added 100 c.c. of NaOH (specific gravity 1.145) as above. Then the whole is filled up to 1 litre.

Twenty cubic centimetres of this reagent are diluted with 75 to 85 c.c. of water. The dilution depends somewhat on the percentage of sugar in the diluted urine; the smaller the amount of sugar the less the dilution. The diluted reagent is heated to the boiling point and titrated with urine. From 1 to 3 c.c. of the urine should be allowed to flow in *at once* and the mixture boiled for half a minute after each addition of urine. As soon as approximately enough urine has been added to reduce all the mercuric cyanide, the mercury (and the phosphates) settle to the bottom and the liquid becomes clear. The reaction is complete when the supernatant fluid contains no more mercury salt. In order to determine the absence of the latter, a number of reagents have been recommended (viz., ammonium sulphide by Knapp, the originator of the method; sulphureted hydrogen by Leussen, and stannous oxide solution by Brumm). The writer has found the last-named indicator to be the best and uses it exclusively. The alkaline solution of stannous oxide is prepared by dissolving 50 gm. of stannous chloride in soda lye and diluting the solution to 1 litre. This reagent does not keep for a long time, as the stannous salt is soon oxidized. The addition, however, of a little metallic tin prevents this oxidation and preserves the reagent indefinitely. A drop of the clear supernatant fluid described above is placed on a porcelain dish and a drop of the stannous chloride solution added. If the fluid still contains mercury the drop turns gray; if it does not, it remains clear.

The calculation of the sugar percentage of the urine to be tested is based on the composition of Knapp's reagent; for the latter is compounded so that 20 c.c. correspond to 0.05 gm. of glucose. The calculation is similar to the one outlined above under "Fehling's test."

The Method of Pavy.—By the addition of ammonia to Fehling's solution the cuprous oxide formed in Fehling's test is held in solution, and the color change indicating the end-reaction is consequently not masked by the precipitate in suspension.

Pavy adds 300 c.c. of ammonia (specific gravity 0.880) to 120 c.c. of Fehling's solution, and fills the mixture up to 1 litre. Of this solution, from the Fehling's solution which it contains, 1 c.c. should indicate 0.6 mgm. of glucose. In reality, however, it only indicates 0.5 mgm. In other words, it is exactly 0.1 time as strong as Fehling's solution. The solution can be kept indefinitely. The above error, according to a later communication by Pavy, can be corrected by dissolving 20.4 gm. of potassium hydrate, 20.4 gm. of Rochelle salt, 300 c.c. of ammonia (specific gravity 0.880), and 4.158 gm. of copper sulphate in 1 litre of water; of this mixture 1 c.c. = 0.5 mgm. of sugar or 10 c.c. indicate 5 mgm. of sugar as the original Fehling's solution.

The titration should be performed with exclusion of the air. A small flask holding 80 c.c. is closed with a stopper with two perforations. Through the one hole passes the burette, through the other a U-tube filled with pumice stone that is saturated with dilute acid in order to bind the escaping fumes of ammonia. Ten cubic centimetres of the reagent are diluted with an equal volume of water and boiled for a few minutes until all the air is driven out of the flask. Then the diluted urine is allowed slowly to flow into the boiling reagent until discoloration is complete. This method is very convenient, very easy of execution, and sufficiently accurate to merit the universal popularity that it enjoys.

Other methods of quantitative sugar determination by titration have no advantages over the three described. They are, therefore, omitted.

By Fermentation. (*a*) Direct Determination.—With the aid of a saccharometer (see above), provided with a scale, the percentage of sugar may be read off directly, the amount of carbonic-acid gas developing during a stated time, at a certain temperature, and in urine diluted to a certain strength, indicating the amount of sugar disassociated by the yeast. The results of this test, even in complicated apparatus, are very inaccurate, for the sources of error are many. For clinical purposes of comparison, Einhorn's saccharimeter is nevertheless a useful instrument.

(*b*) Differential Density Determination before and after Fermentation. — The density of the urine before and after fermentation is determined and the difference in the densities multiplied by an empirical factor: the latter is obtained by dividing the figures for sugar (obtained by some other method) by the difference in the densities of such a stated sugar solution of known percentage before and after fermentation. It is known empirically that a diminution in the density of 0.001 corresponds to the loss of 0.234 per cent. of sugar by fermentation. In order to accelerate fermentation certain nutritive salts may be added to the urine. The best addition is a mixture of 2 gm. of Rochelle salts and 2 gm. of diacid potassium phosphate to each 100 c.c. of the urine; on addition of 10 gm. of compressed yeast to 100 c.c. of urine prepared in this way fermentation (at 30 to 34 c.c.) is usually complete within three hours. Before determining the specific gravity the suspended yeast cells should be precipitated by lead chromate; 100 c.c. of the urine are treated with 10 c.c. of a solution of neutral potassium chromate, saturated in the cold, and 10 c.c. of a solution of lead acetate. For purposes of comparison the original urine should be treated with the same precipitating mixture and the same nutritive salts, as the presence of these salts naturally changes the specific gravity. It has been found empirically, however, that the addition of 0.0178 to the specific gravity of the original urine corrects this error. The density determinations are best performed with the pyknometer, as aerometers do not, as a rule, give readings that are accurate for a sufficient number of decimal points. The difference in the specific gravities multiplied by 0.234 indicates the amount of sugar in per cent. If the urine contains more than 0.5 per cent. of sugar, this method yields excellent results. When less than 0.5 per cent. is present, the method is not so accurate.

Polarimetric Determination. See under qualitative tests; for the details I refer to text-books of chemistry and physics.

Levulose.—Two levuloses occur in human urine, *i.e.*, *fructose* and *laiose*. The former is the more important of the two.

Levulosuria (Fructosuria).—In the older literature a few isolated cases are on record of dextrosuria in which at the same time a small quantity of levulose was excreted. Quite recently Rosin and Labaud (*Zeitschr. f. klin. Med.*, 1902, vol. lxxiv, p. 182) have shown that considerable quantities of levulose are excreted in a large proportion of cases of diabetes, and that in addition there is a group of cases in which levulose alone is excreted, or in which only minimal quantities of dextrose are excreted together with large quantities of levulose. In the latter group of cases there is at the same time levulosuria.

The presence of levulose is not easy to detect if the urine at the same time contains much dextrose. The characteristic features of such urine are the following: The urine in the first place must give the so-called Seliwanoff reaction (see below). This indicates the presence of levulose (and of certain rare ketoses that need not be considered clinically). The reaction of the urine must be acid (diabetic urine that has not undergone decomposition usually *is* acid), for in alkaline urine dextrose seems spontaneously to undergo metamorphosis into levulose and mannose. In alkaline urine, therefore, the value of Seliwanoff's reaction as an indicator of levulose is impaired.

Seliwanoff's Reaction for Levulose.—Equal parts of urine, fuming hydrochloric acid, and a few grains of resorcin are rapidly heated; a deep red color appears and a dark precipitate forms that is soluble in alcohol with a bright red color.

In order to determine, in the second place, the proportion of levulose to dextrose the following plan may be pursued: The sugar of the urine is, first, determined by use of the reduction tests, and, second, by polarization. If there is a considerable difference in the amount of sugar indicated by these two tests, if, *e.g.*, more sugar is indicated by reduction than by polarization, then the urine is subjected to fermentation until no rotation can be obtained and until the reaction of Seliwanoff is negative. If the reducing power of the urine disappears at the same time, then the difference between the figures obtained from reduction and polarization of the original urine must be attributed to the presence of considerable quantities of levulose, for no other urinary substance excepting fructose (levulose) is fermentable and gives Seliwanoff's reaction at the same time.

The following example (quoted from Rosin and Labaud, *loc. cit.*) may illustrate the method:

Case IV.—Urine gives marked Seliwanoff reaction. Titration (Fehling) indicates 7.1 per cent., polarization 6.4 per cent. After fermentation: Seliwanoff reaction negative, polarization 0. Titration: The urine contains less reducing substance than would correspond to 0.1 per cent. dextrose. Difference between titration and polarization, 0.7 per cent.

The presence of levulose in many cases of diabetic urine may aid in explaining the well-known fact that titration of diabetic urine so frequently indicates higher values for sugar (dextrose) than polarization. We must imagine that the tendency of the dextrose to rotate the plane of polarized light to the right is in part neutralized by the opposite tendency of the levulose present in such urines to rotate it to the left.

Other reducing bodies like pentose and glycuronic acid occur in too small quantities to impair the validity of the viewpoint enunciated above. Urines, moreover, that do not give Seliwanoff's reaction do not reveal the differences described. Levulose, therefore, is in all probability the chief and only cause. Rosin and Lebaud succeeded in strengthening this view by manufacturing levulose as such from urine giving the above tests (they employed the method of C. Neuberg, *i.e.*, they precipitated the levulose with methyl-phenylhydrazin as a methyl-phenylosazon).

Other methods have been proposed to determine the presence of levulose in diabetic urines; but all of these are complicated, and, moreover, ambiguous; none of them gives results that are so conclusive as the above method, so that they will not be discussed in this article.

Laiose (Syn: Leo's Sugar). This sugar was found by Leo in three out of twenty-one diabetic urines. Here, too, titration revealed from 1.2 to 1.8 per cent. more sugar (calculated for dextrose) than polarization. In order to identify this interesting sugar it must be isolated from the urine as follows:

The urine is treated with lead acetate and the lead precipitate filtered off; the filtrate is treated with ammonia; the precipitate contains laiose and dextrose. It is washed out with water and decomposed with H_2S; the lead sulphide is filtered off and the filtrate evaporated to a syrupy consistence in the vacuum over concentrated H_2SO_4. The syrupy residue is dissolved in methyl alcohol and the dextrose precipitated by a solution of barium hydrate in methyl alcohol. So much of the reagent should be added that the liquid becomes alkaline. The mixture is filtered, the ammonia removed by placing the dish under a bell jar or over H_2SO_4. This leads to the precipitation of barium carbonate and some barium dextrose. The barium is removed from the filtrate by CO_2. The methyl alcohol is removed by distillation in vacuo; the residue

is dissolved in water. It contains any laiose that may be present.

Laiose is distinguished from other sugars by the following properties: It is not so sweet as dextrose; has smaller reducing powers, and does not ferment so actively. It has a specific rotatory power, and it forms a compound with phenylhydrazin. Although laiose possesses all these properties that seem to class it with the sugars, it is not identical with any known sugar. In fact, it appears doubtful whether it is a hexose or a pentose. (See also Leo: *Virchow's Archiv*, vol. cvii., p. 108, 1887).

Disaccharides.—Isomaltose has been isolated from the urine in small quantities. It is precipitated with other carbohydrates as a benzol ester when the urine is treated with benzoyl chloride (see above, page 38). It is possible that it is formed from dextrose in process of isolation. This sugar is clinically altogether unimportant.

Lactose (*Sugar of Milk*).—This sugar is found in small quantities in the urine of nursing women if there is stasis of milk ("lactosuria"). After the ingestion of 100 gm. of lactose this sugar may appear in the urine of men, or of women who are not nursing. On an abundant milk diet lactose may also appear in the urine. In diabetics the ingestion of lactose leads to the excretion of dextrose. Inversely in nursing women excessive quantities of dextrose (150 gm.) may lead to the excretion of lactose. In new-born children with gastric catarrh lactose has been found in the urine.

Tests. Rubner's Test.—The urine is boiled for two or three minutes with an excess of lead acetate. If lactose is present, the liquid turns yellowish-brown; the precipitate that forms is dissolved in a little ammonia. The liquid now turns brick-red. On standing, a cherry-red or copper-colored sediment settles at the bottom of the tube, while the supernatant fluid becomes clear and colorless. If dextrose is present, this test cannot be applied because a similar reaction is obtained with this sugar. Sometimes lactose and dextrose can be differentiated by heating the urine gently to about 80° (not to boiling). If lactose alone is present, the liquid will not turn red, but yellow or brown, whereas if dextrose alone is present it will turn red.

Fermentation Test.—Lactose does not ferment, but it reduces metal oxides. If, therefore, the urine after fermentation still possesses reducing powers, the presence of lactose may be suspected if the case is a woman in the puerperium, or if the subject is living on a milk diet. The test is by no means, however, conclusive, for lactose, as other non-fermentable sugars, notably pentoses, may be present. In order fully to identify lactose it must be isolated from the urine (see Hofmeister, *Zeitschr. f. phys. Chem.*, vol. i., p. 105).

Polysaccharides.—Animal Gum (Syn.: *Achroglycogen, Urinary Dextrin*). Animal gum is always present in the urine. It is precipitated as a benzol ester with benzoyl chloride (see page 38.) In certain chronic disorders animal gum is increased. Together with isomaltose (that is also precipitated by benzoyl chloride) urinary dextrin forms the group of *unfermentable urinary carbohydrates*, a class of bodies that are attracting much attention and that promise to attain considerable clinical importance.

Animal gum can be isolated from the benzoyl chloride precipitate that forms in a mixture of the urine and sodium hydrate by a complicated method that is described in detail in Huppert's "Harnanalyse," p. 144, 1898. The body is not of sufficient interest for the present to warrant a full description of this method in an article of this limited scope.

Glycogen (Erythrodextrin?). In the urine of diabetics a body is occasionally found (particularly after the dextrose has been caused to disappear) that gives a brown color with iodine. Such urine reduces Fehling's solution only after prolonged boiling. This body has so far never been found in the urine of healthy subjects. From the material published so far it is impossible to determine whether this body is glycogen or erythrodextrin. On physiologic grounds presumptive evidence is in favor of the former supposition.

To test the urine for glycogen (erythrodextrin) a first specimen should be treated with several volumes of alcohol. The alcoholic precipitate, if the urine is diabetic, should be freed from sugar by repeated washing with alcohol or by repeated solution in water and reprecipitation with alcohol. Certain portions of the precipitate, if glycogen is present, assume a brown tint if treated with iodine. By boiling the precipitate for half an hour with ten-per-cent. H_2SO_4 any glycogen or erythrodextrin that may be present is hydrolyzed and converted into dextrose, and the latter, after neutralization of the liquid with sodium hydrate solution, will reduce Fehling's solution, give a phenyl glucosazon, and (after removal of salts by dialysis) ferment (method of Leube).

Aromatic Constituents.—The aromatic constituents of the urine are derived from the degradation of albumins. These degradation products may either enter the body preformed in the food, or they may be formed in the gastro-intestinal tract by bacterial putrefaction, or they may be formed in the tissues proper with or without the intervention of bacteria. Occasionally these aromatic bodies appear in the urine as such. As a rule, however, they appear in combination with sulphuric acid, glycuronic acid, or glycocoll, so that we can conveniently subdivide the combined aromatic constituents of the urine into three groups, viz.: (1) *the conjugate sulphates*, (2) *the compound glycuronates*, (3) *the compound glycocolls*. A fourth group of aromatic bodies, *i.e.*, the aromatic oxyacids, more frequently occurs as such and not combined, so that these substances will be discussed separately under the heading of (4) aromatic oxyacids.

(1) *Conjugate Sulphates.*—The conjugate sulphates include the following bodies: Phenols (kresol, pyrocatechin, hydroquinon), indol (indican), and skatol. Of these bodies the majority are eliminated as the sodium or potassium salt of the ethereal sulphates (*e.g.*,) ($HO.C_6H_4.SO_2OK$). Indol and skatol, however, are not eliminated as indol or skatol sulphuric acid salt, but undergo preliminary oxidation to indoxyl and skatoxyl, and consequently appear in the urine as indoxyl sulphuric acid (indican) or as skatoxyl sulphuric acid. The most important member of this group is *indican*. The amount of ethereal sulphates excreted in man varies according to the character of the food, the ingestion of certain drugs that are excreted as ethereal sulphates (carbolic acid, *scil.* phenol, etc.), the degree of putrefaction going on in the intestine, the character of the putrefactive bacteria, and the existence of certain morbid conditions of the system that lead to perverted tissue metabolism. The total average quantity of conjugate sulphates approximately equals 0.1 of the mineral sulphates, *i.e.*, on an ordinary mixed diet from 0.15 to 0.3 gm. of SO_3. We do not know where in the body the synthesis of the aromatic conjugate sulphates occurs.

The presence of aromatic ethereal sulphates can be detected as follows: Not less than 25 c.c. of urine are acidulated with acetic acid and the mineral sulphates precipitated with barium chloride. The precipitate of barium sulphate is filtered off and the filtrate heated with hydrochloric acid. As the ethereal sulphates are not precipitable by barium chloride and as they can be decomposed by heating with hydrochloric acid, the mixture now contains ordinary sulphuric acid and alcohols derived from the splitting of the ethereal sulphates. In this liquid the liberated sulphuric acid can be precipitated by barium chloride, so that the appearance of a barium sulphate precipitate speaks for the presence of conjugate sulphates in the original urine. This method can also be employed for the quantitative determination of the conjugate sulphates (see paragraph on "Sulphates" under Inorganic Constituents).

Phenols.—The most important of the phenols is parakresol. Phenol itself is less abundant, and pyrocatechin and hydroquinon are excreted only in very small quantities.

Phenol.—Phenol given by mouth appears as conjugate sulphate. If more is given than can be combined with