III.—CLINICAL URINARY ANALYSES.

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As it has been suggested to me that fuller notes on the detail of methods employed in quantitative work might be useful to any interested in the subject, or likely to take it up, I have put together from my notebooks the methods which have been found the most practical and simple, yet of sufficient accuracy for all clinical purposes. The notes have been gleaned from many sources, and considerable time and trouble has been expended in the selection of methods.

Until quite recent years quantitative urinary analysis has been too complicated and laborious to be undertaken by any but the analytical chemist or those engaged in researches on chemical physiology and pathology. Now, however—chiefly abroad—great attention is being paid to quantitative urine work, as its importance is being recognised. I have worked with a view to helping in the process of simplifying quantitative methods, believing that with simple yet reliable methods will come the workers who are most wanted—the doctors in charge of cases. None of the methods given in the paper are long except three—those for the sulphates, uric acid and purin bodies; and these three methods will, I hope, shortly be abandoned for one of the centrifugal methods foreshadowed in this paper. I hope when the necessary tables for these processes have been completed that it will be possible to finish a complete quantitative urinary analysis in one hour, including the estimation of the total nitrogen, extracts, acidity, urea, uric acid, purin bodies not uric acid, preformed ammonia, phosphates, chlorides, sulphates both preformed and conjugated, and the urobilin.

I venture to think that an hour so spent and results so obtained will by the insight given into the inner working of that complex factor, the human body, repay the labour expended.

Estimation of Urinary Acidity.—The total acidity of urine may be estimated in many ways; but as the results obtained
by different procedures differ considerably, whichever method is adopted should be adhered to for all analyses.

The simplest method is to titrate a given quantity of urine, say 20 c.c., with a decinormal alkaline solution, till a drop of the titrated urine turns neutral litmus paper slightly blue. The number of c.c. of decinormal solution used, say x multiplied by \( \frac{100}{5} \), and by \( \frac{1}{5} \), i.e., by 5, will give the amount of urinary acidity per litre of urine in terms of normal alkaline solution.

A more satisfactory and still simple method is that introduced by Folin; it is based on the principle that phenolphthaleine, which while otherwise an unsatisfactory indicator in estimating urinary acidity, owing to its slow end reaction in the presence of ammonia salts in the latter, is rendered an eminently satisfactory one when these are removed by the addition of an oxalate of potassium.

The oxalate of potassium also precipitates any calcium salts present, which, by combining with the \( \text{P}_2\text{O}_5 \) to form basic phosphates and liberating free phosphoric acid, increase urinary acidity. To determine the total acidity, place 25 c.c. of urine in a flask (dilute if the urine is highly coloured), add a few drops of phenolphthaleine, and from 15 to 20 grms. of oxalate of potassium, agitate the flask well and titrate at once with a decinormal alkaline solution till a faint rose colour is obtained. The number of c.c. of decinormal alkaline solution used, multiplied by 40 or by 4, will give the amount of urinary acidity in terms of decinormal and normal alkaline solutions respectively.

The Extract.—The urinary solids may be estimated by evaporating and drying a small quantity of the urine over a water-bath, and then weighing; but this is a long and laborious process, and a much simpler and perhaps equally accurate one is to take the specific gravity in a specific gravity bottle or Westphaal’s balance (urinometers are hopelessly inaccurate), and multiply the last two figures by Häsers’s coefficient (2.33) to obtain the solids per litre.

The Westphaal balance (fig. 1) is based on the principle that a body immersed in a liquid loses a part of its weight equal to the weight of the displaced liquid.

The apparatus consists of a beam balanced on a stand. The free end of the beam has a hook from which a glass plummet is suspended; the other end of this beam ends in a point, which when the machine is regulated is exactly opposite a projecting point on the stand. The upper edge of the beam is divided by notches into a graduated scale, on which various-sized riders representing hundred, ten and unit weights respectively may be placed. The balance, with the ten weight placed on the hook at the end of the beam, is balanced exactly if distilled water is placed in the glass, the head of the plummet then remaining just immersed in the water.
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If urine be placed in the glass instead of water the balance will be upset, and riders of various sizes will have to be placed on the notches of the beam to redress it. The size of the riders required and the notches in which they will have to be placed to restore the balance (i.e., bring the two points shown in the picture opposite each other) will give the specific gravity of the urine.

The balance is standardised for a temperature of 15° C., and Purdy recommends that one figure should be added to the specific gravity found for every 7° of temperature above 15° C.—say specific gravity at 22° C. is 1019 : . true specific gravity is 1020. The last two figures, multiplied by 2·33 (20 × 2·33), equals 46·6, the amount of solids present per litre of urine.

![Westphaal's Balance](image)

N.B.—For this Figure, as well as for figs. 2, 5 and 6, I am indebted to Messrs. Baird and Tatlock, of Cross Street, Hatton Garden, who very kindly lent me the woodcuts.

The Total Nitrogen.—The simplest and most practical method of estimating urinary nitrogen is by the well-known Kjeldahl's method, which is based on the principle that if strong sulphuric acid be added to a nitrogenous fluid and heated, ammonium sulphate, carbonic acid and water are formed. The ammonia can be recovered from the ammonium sulphate by distillation, and estimated. From the amount of the NH₃ present the N. can easily be calculated.

Procedure.—To 5 c.c. of urine in a long-necked flask add 10 c.c. of strong sulphuric acid and 5 c.c. of a 30 per cent. solution of neutral oxalate of potassium (which hastens the action of the sulphuric acid on nitrogenous matter). Place a balloon-shaped stopper in the flask mouth to prevent loss of the sulphuric acid fumes formed on heating the flask. Heat at first moderately and afterwards strongly, till the fluid in the flask is decolourised. The flasks used for boiling are seen on the right-hand side of the accompanying illustration.

Now cool the flask and add distilled water slowly, to 200 or 300 c.c., add a few drops of phenolphthalein or other indicator to the acid fluid,
and then an alkaline solution such as a 20 per cent. caustic soda, till the indicator shows that the fluid is now decidedly alkaline. Attach the flask to a distilling apparatus (such as that seen in the picture), into the receiving flasks of which 50 c.c. of a decinormal solution of sulphuric acid has been placed. Now heat the flask containing the alkaline ammonia solution and allow the ammonia to distill over into the \( \frac{7}{10} \) acid solution. Titrate the acid solution after all the ammonia has distilled over, against a \( \frac{7}{10} \) alkaline solution. The moment when the ammonia distillation is complete may be determined by applying some litmus paper to the tube of the distillation apparatus where it enters the flask containing the sulphuric acid. Each c.c. of \( \frac{7}{10} \) acidity lost by the distillate represents 0.0017 gr. of ammonia and 0.0014 gr. of nitrogen.

Example.—After receiving the ammonia distilled from 5 c.c. of urine, 50 c.c. of a \( \frac{7}{10} \) acid solution were neutralised by 20 c.c. of a \( \frac{7}{10} \) alkaline solution; \( \therefore \) 30 c.c. of \( \frac{7}{10} \) acidity lost; each c.c. = 0.0014 nitrogen; \( \therefore \) 30 c.c. = 0.042 gr. nitrogen in 5 c.c., or \( \frac{0.042 \times 1000}{5} = 8.4 \) grm. nitrogen per litre of urine.

**Fig. 2.**—Kjeldahl’s apparatus. *a*, Distillation apparatus; *b*, boiling flasks.

**Urea.**—The most rapid method of estimating urea has already been described in my first paper. Another method which while not nearly so rapid, is perhaps even more accurate, is that recently devised by Folin. This process depends on the fact that urea when heated along with magnesium chloride breaks up into an ammonium salt;* the amount of the latter

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* N.B.—This break-up of the urea is not due to a chemical union with the magnesium chloride evidently, but to the high temperature at which magnesium chloride boils when dissolved in its water of crystallisation alone (viz., 160° C.), causing a complete break-up of the urea to ammonia.
can easily be estimated, and from it the original amount of urea in the sample calculated.

Procedure.—Three c.c. of urine are placed in a 200 c.c. flask, the rubber cork of which is pierced by a glass condensation tube six or eight inches long. Twenty grms. of magnesium chloride and 2 c.c. of pure hydrochloric acid are added. The flask is now heated gently until the magnesium chloride dissolves in its own water of crystallisation, and then more briskly for ten minutes. Loss of water from the already highly concentrated solution is prevented by the glass tube, which condenses the steam and causes the drops of water so formed to drop back into the boiling fluid. When the drops of water falling back into the flask do so with a hissing noise, the flame under the flask is reduced, and the fluid boiled gently for half an hour. At the end of this time all the urea has been converted to an ammonium salt. After the flask has cooled somewhat, the ammonia is estimated by adding 500 c.c. of distilled water and an excess of alkali (8 or 9 c.c. of a 20 per cent. solution of caustic soda), and distilling the ammonia over into a known quantity of decinormal acid solution, as in the Kjeldahl process just described. The next step is to estimate the amount of acidity lost in the decinormal acid solution through the presence of the ammonia by titrating this with a decinormal alkaline solution. Before doing so, however, the acid-ammonia solution should be boiled to drive off any carbonic acid brought over by the distillation.

As by this method the preformed ammonia is included in the result, it must be eliminated by (1) estimating the preformed ammonia by Shaffer’s process and deducting its amount from the amount found here; or (2) precipitating the ammonia along with other urinary nitrogenous bodies by aid of a 10 per cent. solution of phosphotungstic acid. If very accurate results are required the latter method is probably the best, as undoubtedly small amounts of the creatinin and other extractives appear to be broken up to ammonia by the action of the magnesium chloride and high temperature. Each c.c. of decinormal acid solution neutralised by the ammonia distilled over represents 0’0017 gr. of N\textsubscript{2}H\textsubscript{4}, 0’0014 gr. of nitrogen, and 0’0060 gr. of urea.

Example.—The ammonia given off by the 3 c.c. of urine neutralises 9 c.c. of decinormal acid solution: \therefore 1 c.c. would neutralise 3 c.c. of decinormal acid solution: \therefore 1,000 c.c., or 1 litre, would neutralise 3,000 c.c. of decinormal acid solution. Now 1 c.c. of decinormal acid neutralised = 0’0017 gr. ammonia and 0’00060 gr. urea: \therefore the amount of ammonia and urea per litre of urine would be 0’0017 \times 3,000, or 5’1 grammes ammonia, and 0’0060 \times 3,000, or 18 grm. urea respectively.

Suppose the preformed ammonia has been estimated by the Shaffer process to be 567 grm. per litre (which is equal to 2 grm. urea), \therefore the amount of urea alone in the 3 c.c. of urine will be 16 grm. per litre. If the urine has been previously acted upon by phosphotungstic acid, which precipitates the ammonia, this deduction will not be necessary.

Note.—A method recommended by Folin, but which I have not as yet tried, might be adopted to estimate both the urea and the preformed ammonia from the one operation. This is rendered possible by the fact
that the preformed ammonia of the urine appears to distil over before the ammonia salt formed by the magnesium chloride and heat method just described, in fact, is completely distilled over in the first forty-five minutes. Moreover, the rate of distillation of the ammonia derived from the urea is constant. Now if the distillation be divided into two periods of forty-five minutes each (water being added before the second distillation to replace the same amount lost by the first distillation), it is evident that the amount of ammonia distilled over in the first distillation, minus the amount of the second, will give the preformed ammonia of the urine operated on, and this amount when subtracted from the whole ammonia distilled over will give the NH₃ of urea.

Uric Acid and the Urates.—The most rapid and practical volumetric method of estimating the total urates present (i.e., the uric acid + the purin bodies) is that of Haycraft Deroide as modified by Denigès.

This method depends on the fact that if a mixture of magnesium and silver salts in ammoniacal solution be added to urine the urates present take up some of the silver-magnesium mixture, forming a double urate with these metals, which is precipitated. Denigès, instead of estimating the amount of silver, and hence urates, directly from this precipitate—a long and laborious process used in the method of Haycraft Deroide—estimates the amount of silver left in the filtrate after precipitation has taken place. As the strength of the silver in the silver magnesium mixture used is known, as well as proportions in which the urate and silver combine, an estimate of the amount of silver left in the filtrate after precipitation will give the amount of silver taken up by the urates, and hence the quantity of the latter present. The reason why an ammoniacal solution of silver and magnesium is used is that the ammonium present prevents the urinary chloride taking up the silver.

The amount of silver present in the urine filtrate (after removal of the precipitated urate of silver) is estimated by adding a quantity of a cyanide of potassium solution equivalent to the amount of silver originally added to the urine. When equal quantities of equivalent strengths of potassium cyanide and silver nitrate are brought together, a solution of a soluble double cyanide of silver and potassium is formed in which there is no excess either of silver or of cyanide. Owing to the removal by the urates of some of the silver solution originally added to
the urine, a cyanide solution of equivalent strength to the original solution, if now added to the filtrate, must produce an excess of cyanide in the mixture. This cyanide excess is a measure of the silver taken up by the urates in the silver urate precipitate; it can be estimated by adding a silver solution of known strength till the excess is neutralised. The saturation of the cyanide solution by the silver is easily estimated, as any excess of silver forms an insoluble precipitate of silver iodide, if a little potassium iodide be previously added to the cyanide filtrate mixture.

Procedure.—Two solutions are prepared. Solution A is prepared by adding together equal parts of (1) an ammonia-magnesium mixture, and (2) a decinormal solution of silver.

The magnesium mixture (1) is made by dissolving 150 grms. of chloride of ammonium and 100 grms. of chloride of magnesium in three-quarters of a litre of strong ammonia in a flask. The mixture is then corked and warmed to 25° or 30° C. (under a warm water tap) and the volume completed to the litre. The decinormal silver solution (2) is made by dissolving 17 grms. of silver nitrate in a litre of distilled water. The ammoniacal silver-magnesium solution thus formed keeps well even in white bottles. The strength of the contained silver is \( \frac{3}{20} \).

Solution B is a decinormal solution of potassium cyanide and is prepared by dissolving 17 to 18 grms. of potassium cyanide in distilled water. This solution is then titrated against decinormal solution of silver, and diluted till 10 c.c. exactly neutralises 10 c.c. of silver solution, neither salt being in excess. In addition to the above solution, a 20 per cent. solution of potassium iodide is made up as an indicator to show when saturation of the cyanide solution by the silver has taken place. The least excess of silver present in this case is indicated by the formation of an insoluble white precipitate of silver iodide.

To 100 c.c. of urine add 25 c.c. of solution A (the ammoniacal magnesium-silver solution), filter off the precipitate of urate of magnesium and silver formed. Take 100 c.c. of the filtrate representing 80 c.c. of urine \( \left( \frac{100}{125} \text{ of } 100 = 80 \right) \), and 20 c.c. of the \( \frac{3}{20} \) silver solution, and to this add 10 c.c. of solution B (potassium cyanide solution). Having added a few drops of the indicator (potassium iodide), the decinormal silver nitrate solution is dropped in from a burette, till a persistent cloudiness is obtained. Each c.c. of silver solution used represents 0.0168 grms. of urates expressed as uric acid; or to make it simpler still, multiply the c.c. of silver solution used by 21, and the answer will represent the quantity of urates present per litre of urine.

Example.—To the 80 c.c. of filtrate obtained from the mixture of 100 c.c. of urine, add 25 c.c. of ammonia-magnesium silver mixture, 10 c.c. of cyanide solution are added. The \( \frac{3}{20} \) silver nitrate solution necessary to neutralise the excess of cyanide present is 3.2 c.c.

Each c.c. of silver nitrate \( \frac{3}{20} \) solution equals 0.0168 grms. of urate expressed as uric acid is 80 c.c. of urine: \( \therefore 3.2 \text{ c.c. equal } \frac{0.0168}{3.2} \text{ grms. uric acid in } 80 \text{ c.c.}, \text{ or } 0.0376 \times \frac{80}{3.2}, \text{ or } 672 \text{ grms. in } 1 \text{ litre} \) (which is the same amount as multiplying 3.2 by 21).
Uric Acid alone.—There are a great many methods employed for estimating the uric acid of urine, but those that are very accurate are very laborious, and others that are quicker are not very accurate. Of the longer methods that of Hopkins and its modifications are the best and simplest; but I have found the copper process of Denigès to be both accurate and rapid. It has been adopted as the standard method in France.

In this method, and in Blarez’ modification of it, the uric acid is precipitated from the urine as urate of copper, and the copper in the precipitate estimated. The combining proportion of urate of copper being known, the amount of uric acid is readily estimated when the copper in the combination has been calculated.

The phosphates present in the urine are first eliminated by the addition of a 16 per cent. solution of sodium carbonate. A colourless alkaline copper solution (made by decolourising some Fehling’s solution with a little alkaline bisulphite) is now added. The uric acid of the urine is taken up by the copper, and an insoluble urate of copper precipitates out. The precipitate is collected on a filter and thoroughly washed with hot water. Collecting the precipitate by filtering is a tedious process unless suction is applied to the filter. (If a water-pump be used the filtering is very rapid and satisfactory results are obtained.) The urate of copper combination has now to be broken up and the amount of copper in the precipitate estimated. These processes may be carried out in one of two ways.

Denigès breaks up the urate of copper by putting the precipitate into a capsule and adding \( \frac{1}{2} \) to \( 1\frac{1}{2} \) c.c. of hydrochloric acid, and heating. Blarez adds 10 c.c. of a 50 per cent. solution of sulphuric acid and uses a flask. Denigès estimates the amount of copper present by first adding 10 c.c. of ammonia to form a bright blue combination (copper salts in the presence of \( \text{NH}_3 \) are blue), and then decolourising this blue fluid by a decinormal solution of potassium cyanide (compounds of alkaline cyanide with copper are colourless).

It is evident from this that the amount of cyanide solution used will indicate the amount of copper present in the precipitate, and hence the uric acid.

Blarez estimates the copper in the precipitate by adding a decinormal solution of permanganate of potassium to the copper
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sulphate solution until the appearance of a faint rose colour* which persists for one minute.

Solutions required for Deniges' Method.—(1) Sixteen per cent. solution of anhydrous carbonate of soda. (2) Fehling's solution, to which a solution of sodium bisulphite has been added, till the Fehling fluid is decolourised. (3) Hypobromite of soda solution, such as that used for the urea estimation. (4) A \( \frac{5}{6} \) solution of cyanide of potassium similar to that prepared for the estimation of the total urates.

Procedure.—Place in a measured glass 120 c.c. of urine and 21 c.c. of solution (1); filter off the precipitate of phosphates formed. To 100 c.c. of filtrate, equal to 90 c.c. of urine, add 10 c.c. of the decolourised Fehling's solution; filter, and receive the copper urate precipitate on a small flat filter paper (an exhaust pump will be found of great use in accelerating the filtration, which is otherwise long and tedious). Thoroughly wash precipitate with hot water and then wash it off into a porcelain dish; add I to I\( \frac{1}{2} \) c.c. of pure hydrochloric acid and hypobromite solution drop by drop till the copper solution is of a yellowish tint. The total volume of washings should not exceed 40 c.c. Boil, add 10 c.c. of ammonia, which colours the solution deep blue, and when the boiling is brisk drop in the \( \frac{5}{6} \) cyanide of potassium solution till the blue colour has disappeared. The number of c.c. of cyanide solution used, minus 0-01, multiplied by 11, gives the amount of uric acid in each litre of urine.

Solutions used and Procedure in Blarez' Modification.—(1) Sixteen per cent. solution of anhydrous carbonate of soda. (2) Fehling's solution, decolourised by addition of an alkaline bisulphite. (3) \( \frac{5}{6} \) solution of permanganate of potassium.

Procedure.—To 37 c.c. in a measured glass add 5 c.c. of solution 1; add to the mixture, after shaking, 7 c.c. of the decolourised Fehling's solution. After five minutes filter, receiving the copper urate precipitate on a small filter paper. Thoroughly wash the precipitate two or three times. Place the precipitate and filter paper in a flask along with 150 c.c. of water, shake to free the filter paper, add 10 c.c. of a 50 per cent. solution of sulphuric acid, shake well, now add the permanganate solution till a rose-coloured tint persists for half a minute to a minute.

The number of \( \frac{5}{6} \)th of permanganate solution employed multiplied by 2 gives the number of centigrammes of uric acid contained in a litre of urine.

The Method of Estimating the Urinary Chlorides is practically the same as that employed for the chlorides in water (Mohr's method). It is not advisable, however, to act directly on urine, owing to (1) its colour rendering the end reaction difficult to perceive, and (2) the fact that there are other bodies

* N.B.—The permanganate solution gives off oxygen to the uric acid in the solution, and is thereby reduced and decolourised. The reappearance of the red colour of the permanganate indicates that the uric acid is oxygen saturated. The oxygen absorbing power of uric acid being known, its amount can be calculated.
beside the urinary chlorides which affect the titrating silver solution (viz., organic matters—extractives and albumens). The colour difficulty is easily got rid of by diluting the urine, while the organic matter may be destroyed by means of permanganate of potassium in the presence of an acid. An alkali (pure carbonate of lime) must be added to neutralise the urine again, as acidity vitiates the process. The principle on which Mohr's process is based is, of course, that if a chromate be added to the fluid to be analysed and a solution of silver dropped in, the latter is taken up by the chlorides present and only unites with the chromic acid to form a red chromate of silver when the chlorides are exhausted.

Procedure. — To 7·1 c.c. of urine, well diluted, add 2 c.c. of weak sulphuric acid (\(\text{H}_2\text{SO}_4\) will do), boil gently, add permanganate of potassium till a yellow colour is present (the organic matter is now oxidised). Now add a pinch of carbonate of lime, which will not only neutralise the fluid, but will precipitate any oxalates which may have been formed by the action of the permanganate. Add a few drops of a chromate of potash solution and titrate with a decinormal solution of silver nitrate till a faint red colour is apparent. The number of \(\frac{1}{10}\) c.c. of silver solution used divided by 2 gives the amount of chloride in grammes per litre of urine.

The Phosphates. — The total phosphoric acid in the urine is estimated by the nitrate of uranium method, the result being expressed in terms of \(\text{P}_2\text{O}_5\) (anhydrous phosphoric acid). This process depends upon the fact that if a uranium salt such as the nitrate or acetate be added to urine, it combines with the phosphates present to form a phosphate of uranyl: the amount of uranium salt necessary to saturate the phosphates present is an index of the quantity of the phosphates present in the urine. Saturation of the phosphates is to be considered complete when such an indicator as cochineal or potassium ferrocyanide is attacked by the presence of free uranium nitrate.

The nitrate salt of uranium is not the best one to use for titrating, as free nitric acid is liberated during the titration of the urine. As this result can be completely checked by the addition of a little nitrate of sodium, however, it is better to use this salt than the more expensive acetate of uranium.

Procedure. — The following solutions are prepared:

Solution A. Dissolve 40 grms. of nitrate of uranium in 600 or 700 c.c. of distilled water. As nitrate of uranium often contains free
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nitric acid, this latter must be got rid of by the addition of a little ammonia (which on addition forms a precipitate) and some acetic acid (which dissolves the precipitate formed). As nitrate of uranium is never pure enough to enable a solution of required strength to be made from it directly, solution A has to be corrected by titrating it against a solution containing phosphoric acid in known strength. Such a solution may be obtained by dissolving 3.24 grms. of acid ammonium phosphate, or 5.887 grms. of soda ammonium phosphate, in a litre of water. Fifty c.c. of this solution, containing 0.01 grs. of $P_2O_5$, are titrated along with some cochineal and acetate of soda against the uranium solution; the strength of the latter is now estimated (the most convenient strength of the uranium solution is for 1 c.c. of it to be equivalent to 0.005 grms. of $P_2O_5$).

Solution B consists of 50 grms. each of acetate of sodium and of acetic acid dissolved in half a litre of water.

Solution C is the indicator used (tincture of cochineal).

To 50 c.c. of urine in a porcelain dish add 2 or 3 c.c. of the acetate of soda solution and a few drops of tincture of cochineal. Gently heat the dish (to about 80° C.) and pour in the uranium solution from a graduated burette till a bright green colour is apparent. As each c.c. of uranium solution equals 0.005 grs. of $P_2O_5$, the amount of anhydrous phosphoric acid present in each litre of urine will be found by multiplying the number of c.c. by 0.005 and 20; say 10 c.c. of uranium solution have been used for the 50 c.c. of urine, then $\frac{10 \times 0.005 \times 1000}{50}$, or 1 gr., will be the amount of $P_2O_5$ present per litre of urine.

The Sulphates.—The only urinary sulphur estimations which are clinically practical and of value are those of the total acid sulphates and the conjugated sulphates. While the mineral sulphates may be precipitated by a salt of barium, and estimated as barium sulphate, the phenol sulphates have first to be dissociated by the action of heat and a strong mineral acid before they will yield up their $SO_3$ to a barium salt. This fact permits of the amount of mineral and organic or conjugated sulphates being estimated separately.

Procedure. — Estimation of Total Sulphates. — Boil 50 c.c. of filtered urine along with 5 c.c. of pure hydrochloric acid for fifteen minutes, add 10 c.c. of a hot 10 per cent. barium chloride solution, filter through a small filter paper of known ash; wash the filtrate thoroughly till the washings give no precipitate with a silver nitrate solution; dry and calcine the filter paper and precipitate in a shallow previously weighed platinum dish. A drop or two of nitric acid aids calcination. Now cool the capsule and weigh carefully. The difference in weight between the capsule alone and the capsule + sulphate ash, multiplied by 0.34326, and again by 20, gives the amount of total sulphates in the litre of urine.

Estimation of Conjugated Sulphates.—To 125 c.c. of urine add an equal volume of a mixture of two volumes of barium hydrate and one of
barium chloride solution, both saturated. The precipitate, which consists of the mineral sulphates, is removed by filtration, and 200 c.c. of the filtrate, representing 100 c.c. of urine, boiled for fifteen minutes, along with 20 c.c. of pure hydrochloric acid. The precipitate of sulphates derived from the organic sulphates is collected, incinerated, and weighed, as in the case of the total sulphates, and similarly estimated. The difference between the total and the conjugated sulphates gives the amount of mineral sulphates present.

**Carbon of Urine.**—The carbon contained in a given sample of urine is estimated by breaking up the carbon compounds by means of sulphuric acid and collecting the carbonic acid gas which is evolved in a tube containing caustic potash. A carbon- and moisture-free air is then driven over the urine towards the potash tube to ensure the collection of all the carbonic acid. The method and apparatus used in that of Desgrez, and is as follows: 10 c.c. of urine and 10 grms. of chromic acid are placed in the 100 c.c. bulb marked B., to which are attached by means of a glass stopper three tubes; one tube leads to a stand (A) containing soda lime; a second tube leads up to a bulb (C), into which 25 c.c. of strong sulphuric acid are placed, the acid being allowed to drop slowly into the bulb containing the urine; the third tube leads up to a condenser (D), which is necessary to prevent the CO₂ gas
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passing over too hot and too moist into the rectangular tube E.
The first U-shaped tube (F) contains pieces of pumice stone
saturated with strong sulphuric acid, in order to absorb any
moisture coming over with the evolved CO₂. The second
U-shaped tube (G) contains ferroxyanide of potassium and borate
of soda, which absorb any chlorine or hydrochloric acid which
may have been evolved from the urine. The spiral tube (H)
is filled with caustic potash to absorb the CO₂ of the urine.
Tube I contains pumice stone soaked with more caustic potash,
and serves as a control to tube H; while the last tube (J) is
filled like the first tube (F) with pumice stone saturated with
sulphuric acid. This tube as well as bottle K are used to pre­
vent any moisture getting access to the potash tubes (H and
J) from the water-pump (L).

Procedure.—The potash tubes H and J are detached, weighed, and
re-attached. The 10 c.c. of urine and 10 grms. of chromic acid (which is
necessary to retransform the sulphurous acid formed by the action of
the H₂SO₄ on organic matter into sulphuric acid) are acted on by the
25 c.c. of sulphuric acid gradually introduced from the flask C. The
flask B is then gently heated. The heat applied should be short of
that necessary to cause boiling till towards the end of the operation;
by this means the CO₂ is slowly and steadily driven off from the urine
(it should be possible to count the bubbles of gas as they come off).
When all gas has been driven off a current of air is passed through
the apparatus for twenty minutes; this air is deprived of both carbon
dioxide and moisture by passing through the soda lime (bottle A).
At the end of the operation, which lasts about two hours, the potash
tubes (H and J) are again weighed. The difference between the weights
of the potash tubes (i) before and (2) after the operation indicate the
amount of CO₂ absorbed by the caustic potash, and hence present in
the urine. The amount of carbon is \( \frac{1}{2} \) of this amount.

Ammonia.—Shaffer’s vacuum method of estimating ammonia
was illustrated and mentioned in my first paper on urinary
analyses, but as the details of the process were not given they
are inserted here.

This method consists in driving off the urinary ammonia by
boiling urine in vacuo along with an alkali, which displaces and
drives off the ammonia; the latter is collected in a decinormal
solution of sulphuric acid, and the acidity lost by the acid
determines the amount of ammonia present in the urine. As
the urea of urine gives off ammonia if the temperature exceeds
60° C., the boiling point has to be kept below that temperature;
this is effected by means of the vacuum method and methyl
alcohol. The addition of sodium chloride to the urine also helps to prevent any urea decomposition.

Procedure.—Place in the small flasks (seen suspended in fig. 4) 100 c.c. of a $\frac{1}{10}$ solution of sulphuric acid. In the large flask shown in the water-bath place 50 c.c. of urine, 50 c.c. of methyl alcohol and

20 grms. of sodium chloride; place 3 or 4 grms. of sodium carbonate in the flask and attach this rapidly to the two small glass bulbs seen just above it in the photo. The apparatus used and figured in the illus-

**FIG 4.—SHAPPER'S AMMONIUM APPARATUS.**
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Urine is connected to a water pump and to a mercury gauge. When the urine and other ingredients have been placed in the large flask this is placed in a water-bath, and the rubber tube seen on the right of the photo connecting the flask with the air pinched up. The air in the apparatus is now exhausted by turning on the water pump seen on the left of the photo. When the mercury gauge registers 20 m.m. of mercury the water-bath is heated. Boiling commences usually at or below 40° C. and the temperature must not be allowed to exceed 45° C. The four small glass bulbs seen in the photo above the boiling flask from being drawn into the decinormal acid solutions in the small glass flasks and so spoiling the result. After boiling for twenty minutes a current of air is allowed to pass through the apparatus, the acid-containing flasks are detached, and their acidity determined by titration with a \( \frac{1}{7} \) alkaline solution. Any loss of acidity caused by the ammonia distilled over is determined; each c.c. of acidity lost represents 0.0017 grs. of \( \text{NH}_3 \).

Example.—The \( \frac{6}{7} \) acid of the small flasks when titrated after the process only equals 50 c.c. of \( \frac{6}{7} \) alkaline solution; therefore 100 - 50, or 50 c.c., of \( \frac{6}{7} \) acidity have been neutralised by the ammonia driven off from the urine. Each c.c. of acidity lost, however, equals 0.0017 grms. \( \text{NH}_3 \); therefore, 50 c.c. equals 0.085 grms. \( \text{NH}_3 \) found in 50 c.c. of urine, \( \therefore \) the amount per litre must be \( \frac{0.085 \times 1000}{50} \), or 1.7 grms. per litre.

Sugar.—Of the many volumetric methods of estimating the sugar in urine the best are those of Gerrard and Purdy’s modification of Pavy—both modifications of the well-known Fehling process. In Fehling’s original method—still largely used—the end point of the process is unsatisfactory, as it is difficult to estimate when the blue copper solution has been completely decolourised, owing to the constant formation of the red oxide of copper. Gerrard obviates this difficulty by adding a cyanide to the copper solution, which forms a colourless compound with the copper oxide reduced from the sulphate, thus giving a clear reaction. Pavy’s modification is to produce a similar colourless end reaction with the aid of ammonia (which dissolves the copper oxide precipitate). Purdy has altered the composition of the Pavy-Fehling solution, substituting glycerol for the sodic tartrate employed.

Gerrard’s Process.—Solutions required:—

(1) Fehling’s Solution.—A mixture of equal parts of two solutions: (a) 69.25 grs. of pure copper sulphate, powdered and dried; 1 c.c. of pure sulphuric acid, and water to the litre; (b) 350 grms. of Rochelle salt are dissolved in 700 c.c. of water, 100 grms. of caustic potash are added, and water to the litre.

(2) A 5 per cent. solution of potassium cyanide.
Procedure.—Place 10 c.c. of freshly prepared Fehling’s solution in a porcelain dish and add 40 c.c. of water, heat to boiling, add the cyanide solution carefully till the blue colour of the Fehling is all but gone (excess of cyanide must be avoided). Now add 10 c.c. of Fehling to the faintly blue mixture, and the solution is ready (a stock of this may be made up). These 10 c.c. of Fehling are decolourised by 0.05 gr. of glucose. Dilute the urine to be analysed twenty times and drop it into the boiling copper solution from a burette till the blue colour has completely disappeared: the boiling must be brisk during the whole process.

Say 20 c.c. of urine diluted twenty times decolourises 10 c.c. of Fehling’s solution equal to 0.05 grm. of sugar, ./. the percentage of sugar in the urine is $\frac{20 \times 100}{35} \times 0.05$, or 5 per cent.

Pavy’s Process modified by Purdy.—The procedure here is similar to the above, but the copper solution used is, pure copper sulphate 4.752 grms., potassium hydroxide 23.50 grs., glycerol 38 c.c., strong ammonia 350 c.c., water to the litre. 35 c.c. of this solution are equal to 0.02 gr. of glucose.

The glycerol is substituted for the unstable Rochelle salt used in Fehling’s and Pavy’s solutions. Thirty-five c.c. of the above solution are boiled in a flask, and the diluted urine dropped in from a burette. As it takes 0.02 grm. of glucose to decolourise the 35 c.c., the amount of urinary sugar can readily be ascertained.

Example.—Say 20 c.c. of urine diluted ten times (i.e., 2 c.c. urine) decolourises the 35 c.c. of copper solution, ./. there will be 0.02 grm. of sugar in 2 c.c., or 10 grm. in 100 c.c. = 10 per cent. sugar.

B. Oxybutyric Acid.—The most simple and rapid method of estimating this acid is by the polariscope.

B. oxybutyric acid is levogyric, so by noting the amount of left deviation—when this is present—the amount of the acid can be ascertained. As this acid occurs chiefly in urine containing sugar, the influence of the latter substance, which is dextro-rotatory, must be allowed for. A 100 per cent. solution of B. oxybutyric acid deviates light 24° to the left, while glucose in similar strength deviates light 58.3° to the right (sodium flame). Each degree of a Laurent polariscope with sodium flame is graduated to equal 2.27 grms. of sugar, and 4.64 grms. of B. oxybutyric acid per litre of urine. With a Schmidt and Heusch’s polariscope the amounts of sugar and acid per degree of the instrument are 3.34 and 6.9 grms. respectively. A sodium flame is used with Laurent’s polariscope, and white light with the Schmidt and Heusch.

Procedure.—To estimate the oxybutyric acid in a diabetic urine, in say a Laurent’s polariscope, the following method may be employed. To
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100 c.c. of urine add 10 c.c. of a saturated solution of subacetate of lead; this removes the urinary pigments and clarifies the urine, enabling one to see through a long tube full of it. The 20-c.m. tube of the polariscope is now filled completely with the clarified urine, the tube placed in the polariscope, and the light looked at through the tube and prisms of the instrument. The amount of deviation caused by the sugar and acid together is noted. The amount of rotatory action on light rays caused by a substance is indicated by the nature of the shadow thrown on a finely bisected disc near the eye-piece of the instrument.

Fig. 5.—A cheap form of Polariscope well suited for estimating the sugar in urine. The 20 cm. tube is in position in the instrument. The light used here is a sodium flame.

If a tube of distilled water (which has no polarising action on light) be placed in the polariscope, the shadows on the two sides of the bisected disc are equal; if a dextrorotatory substance in solution, such as sugar, now takes the place of the water in the tube, a dark shadow varying in intensity with the amount of sugar present is seen on the right half of the bisected disc. With a levorotatory substance like B. oxybutyric acid a shadow is thrown on the left half. The amount of rotation present and hence the amount of the substance causing it is estimated by noting the number of degrees through which a quartz disc has to be moved to neutralise the polarisation, and hence restore the shades on the halves of the disc.
The amount of rotation necessary to obliterate the shadow on the right disc, caused by the diabetic urine, being noted, the amount of sugar in the urine is estimated by the cupric method. The amount of deviation caused by definite amounts of sugar being known, it will be evident that if sugar alone is present in the urine, this should be present in amount sufficient to account for the deviation of light shown by the polariscope. If the amount of polaric deviation is less than it ought to be, from the amount of sugar in it, the difference is due to the influence of B. oxybutyric acid, which is strongly levogyric. The difference multiplied by 4.64 gives the amount of B. oxybutyric acid in grammes per litre present in the urine.

Example.—Suppose the 20 c.m. tube full of urine throws a shadow on the right half of the disc which it requires 15° of rotation to obliterate. Suppose, further, the sugar in the urine has been estimated to be 59.4 grms. per litre, as we know that each 2.27 grms. of sugar per litre of urine in a 20 c.m. tube diluted 1/10 equals 1° in a Laurent instrument, .·. there should be 20° of dextrorotation and not 15°; 20° = 15°, or 5° represents the influence of the left rotation of the B. oxybutyric acid. It takes 4.64 grms. per litre of this acid to cause 1° of left rotation in a Laurent polariscope, therefore the amount of B. oxybutyric acid present must be 5° × 4.64, or 23.2 grms. per litre.

More accurate but longer methods of estimating B. oxybutyric acid are: (1) Fermenting the urine with yeast overnight and thus getting rid of any sugar present: the polaric deviation present will be due to oxybutyric acid alone, and its amount will indicate the quantity of B. oxybutyric acid present. (2) If urine is mixed with strong sulphuric acid and the mixture strongly heated and distilled, any oxybutyric acid present is converted to crotonic acid, which is distilled over. Crotonic acid is oxybutyric acid — one molecule of H₂O, and by estimating the crotonic acid formed the amount of B. oxybutyric acid can be determined.

Albumen.—The method which has held the field up to the present for the quantitative estimation of albumen has been the ponderal one. This consists in precipitating urinary albumen by trichloracetic acid, filtering, washing the precipitate of albumen, drying and weighing. While undoubtedly an accurate method, it has no great advantages in this respect over the centrifugal method of Purdy, to be described later on, and is incomparably longer and more difficult. Latterly I have used the centrifugal method alone.

Qualitative Estimation.—Brief details of the methods employed for the qualitative examination of the urine may be of interest. The Albumen has been estimated usually by the nitric acid test, as it yields other information besides the question of albumen. The best method to employ is to place a little urine in a conical urine glass and add the nitric acid by a pipette, the point of which is placed near the bottom of the vessel before the nitric acid is allowed to flow out; the heavier acid floats
the lighter urine up, and a very distinct line of junction is seen: A white haze to band at this junction, signifies albumen, a white ring half inch or more higher up in the urine shows excess of uric acid; a play of colours where the acid joins the urine denotes the presence of bile pigments, and a dark brown band in the same place an excess of urobilin. Boureau's reagent, a mixture of 5 parts of sulphosalicylic and 15 parts of sulphophenic acid in 100 parts of water is a useful and sensitive reagent. The most sensitive test for albumen in the urine is that by Tauret's reagent, which consists of a mixture of 4·06 grms. of perchloride of mercury, 9·66 grms. of potassium iodide, 60 c.c. of crystallised acetic acid, with distilled water to 192 c.c. The reagent gives a precipitate with the albumoses as well as albumen.

Albumoses if present in urine may be detected by first precipitating any albumen present by heat or Boureau's solution, and then adding Tauret's reagent to the filtrate, when, if albumoses are present, a white precipitate will be thrown down.

The test I have usually adopted for indican in the urine is that of Loubiou. To 2 or 3 c.c. of urine add an equal quantity of chloroform, 1 c.c. or so of peroxide of hydrogen, and 3 or 4 c.c. of hydrochloric acid; mix and heat gently. If indican is present a blue colour appears in the chloroform layer of the mixture. The intensity of the colour present shows approximately the amount of indican.

Another similar and excellent method is to substitute sulphuric acid and persulphate of sodium for the hydrochloric acid and peroxide of hydrogen; a larger quantity of urine, 20 c.c., should be taken with 5 c.c. each of chloroform and the persulphate solution, and a few drops of the acid. No heat is required. (Amman's method.)

Bile Pigments have been estimated by the Gmelin-Rosenbach test. It consists in placing a drop of nitric acid on the damp portion of the filter paper through which urine has filtered. Surrounding the spot where the acid has been placed several faintly-coloured rings will appear—yellowish-red, violet, blue and green, in that order, from within outwards.

The most sensitive method of estimating bile pigments is that of Jolles, who has gone carefully into this subject of bile pigment testing.

In a 60 cc. burette place 50 c.c. of urine, a few drops of diluted hydrochloric acid, an excess of barium chloride, and 5 c.c. of pure chloroform. Shake the above mixture well, and leave for ten minutes. Remove the chloroform and precipitate by opening stop-cock (the
chloroform and precipitate are at the bottom of the burette); the small amount of urine usually unavoidably removed at the same time does not affect results. Place chloroform and precipitate in a warm chamber or water-bath for five or ten minutes to evaporate the chloroform. Now add a few drops of nitric acid to the residue—the coloured rings above described are at once seen. This test reveals the presence of bile pigments when in 0·1 per cent. strength, while the Gmelin method unmodified does not give clear results unless 5 per cent. of bile pigments are present in the urine.

**Bile Acids.**—The simplest qualitative test of these acids is that of Hay. Owing to the increased superficial tension given to a fluid by the presence of bile acids, flower of sulphur, which when thrown on a dish containing normal urine floats, will sink rapidly if bile acids are present. Another test is that of Oliver, based on the fact that bile acids precipitate peptones in acid solution.

If to 20 minims of urine 60 minims or 120 drops of a mixture containing 3ss. of peptone, grains 4 of salicylic acid, 5ss. of acetic acid, and water to 3vi. be added, a slight and temporary opalescence is produced if there is no excess of bile acids present in the urine. This method may be used for approximate quantitative work. Appended is Oliver's table.

### Percentage of increase of bile salts over the normal.

<table>
<thead>
<tr>
<th>Urine.</th>
<th>Percentage of increase of bile salts over the normal.</th>
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<tr>
<td>Minims or drops of solution added to 20 minims.</td>
<td>Per cent.</td>
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<tr>
<td>Min.</td>
<td>Drops.</td>
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**Centrifugal Methods** of quantitative estimation. It is convenient to group all centrifugal estimations for the urinary chlorides, phosphates, sulphates, albumens and other constituents of normal or abnormal urine under this heading. To Purdy, of Philadelphia, is due the credit of having first introduced the rapid and satisfactory method of centrifugalisation into urinary work.

Instead of the long volumetric method, or the still longer and more laborious gravimetric method, Purdy essayed to measure the precipitates of chlorides, phosphates and albumen, formed by the addition of suitable reagents to the urine, and
centrifugalised to a compact homogeneous layer in a graduated centrifugal tube.

Purdy's methods are as follows: A centrifugal machine, driven by electricity and capable of a speed of from 1,500 to 10,000 revolutions per minute is employed. The arms of the machine carry tube holders, in which are contained tubes of 15 c.c. capacity, graduated to tenths of a c.c. for the first 10 of the 15 c.c.—the first 5 c.c. being finely drawn out and further graduated in fortieths of a c.c. The radius of the arms and tubes (i.e., distance from the central pivot to the ends of the tubes when held out horizontally) is 6\(\frac{1}{2}\) inches.

To estimate the urinary chlorides, phosphates, sulphates, and albumen (if the latter is present), four of the 15 c.c. centrifugal tubes are filled to the 10 c.c. mark with urine. To No. 1 tube is further added 1 c.c. of strong nitric acid and 4 c.c. of an 8\% per cent. solution of silver nitrate (chlorides). To No. 2, 2 c.c. of a 50 per cent. solution of acetic acid and 3 c.c. of a 5 per cent. uranium nitrate solution (phosphates). To tube 3, 5 c.c. of a mixture containing barium chloride 4 parts, strong HCl. 1 part, and distilled water 16 parts (sulphates). To tube 4, 3 c.c. of a 10 per cent. solution of potassium ferrocyanide and 2 c.c. of a 50 per cent. solution of acetic acid (albumen). The above solutions are allowed to stand for three minutes, till the respective precipitants have formed, are then centrifugalised for three minutes, and the amount of the sediment formed by the various precipitates calculated in bulk percentage to the 10 c.c. of urine employed in each case. Each tenth of a c.c. of sediment thus becomes 1 per cent. bulk percentage; each \(\frac{1}{10}\) c.c. (the first 5 c.c. are divided into fortieths) equals \(25\) per cent. (bulk percentage).

Tables are given by Purdy which show that with the mineral constituents (chlorides, phosphates and sulphates) revolved at 1,200 revolutions of the centrifuge per minute, each \(\frac{1}{10}\) c.c. (25 bulk percentage) of chloride precipitate equals 0.03 per cent. of sodium chloride and 0.02 per cent. of chlorine. The same amount of sulphate precipitate equals 0.06 per cent. of SO\(_4\), while each \(\frac{1}{5}\) c.c. (i.e., \(\frac{1}{5}\) of bulk percentage of the phosphate precipitate) equals 0.005 of anhydrous phosphoric acid (P\(_2\)O\(_5\)), except the first two \(\frac{1}{10}\) c.c., which each represent 0.02 per cent. of phosphates. With the tube containing the albumen precipitate revolved at 1,500 revolutions per minute, each \(\frac{1}{10}\) c.c. (i.e., \(25\) bulk percentage) equals 0.005 per cent. of dry albumen.

The one disadvantage of Purdy's centrifugal method is his insistence on a mechanical (preferably electrical) centrifuge. If this condition were as essential as Purdy considers it, quantitative urinary analysis would be much restricted, as electrical centrifuges are expensive and somewhat difficult to work and manage where electricity is not laid on to a building. I have, however, by experiment and calculation found a means of working out quantitative analyses on the ordinary hand centri-
fuge used largely in laboratories in England, allowing at the same time advantage to be taken of the tables Purdy has drawn up, thus permitting anyone possessing the small "high-speed medical hand centrifuge" to work out quantitative urinary analyses very simply and rapidly, and with an accuracy sufficient for all clinical purposes.

The formula for centrifugal force is \[ C = \frac{v^2 \times w}{r^2 \times 32.2} \]

where \( v \) stands for the velocity (feet per minute covered by the extremity of the centrifugal tube); \( w \) for weight; \( r \) for radius of arms of centrifuge, i.e., length from pivot to tips of extended tubes. The 32.2 stands for gravity. The question to be solved, however, is not the centrifugal pressure at the apex of the tube, but the driving of the particles of the precipitate through the fluid and the conversion of the power of one variety of centrifuge to perform this into terms of another. The problem, therefore, becomes much simplified, weight and gravity can be eliminated from the equation, and the answer obtained by dividing radius of the Purdy centrifuge by that of the instrument which one is using, and multiplying the result by the revolutions used to obtain his results. Thus Purdy obtained his results and formulated his tables of the chlorides, phosphates and sulphates with a centrifuge with 6.5 in. radius, and used a speed of 1,200 revolutions per minute; all that is now necessary is to divide this number (6.5) by the radius of our own machine and multiply the 1,200 by the result.

The standard hand machine used in this country has generally an arm radius of 5 to 6 inches, and a spin of 20 to 60 revolutions to each turn of the handle. The machine I worked with had an arm radius of 5.5 inches, and a spin of 20 to each turn of the handle. Here \( \frac{93}{41} \times 1,200 \) (the revolutions Purdy employed in his chloride, phosphate and sulphate estimations), or 1,560 revolutions, were necessary in order to obtain similar centrifugal force to that which he uses. Purdy employs his centrifugal force for three minutes to obtain his results, so by turning the handle of my machine \( \frac{1560}{78} \), or 78 times a minute, for three minutes, I was enabled to make use of his tables. If a watch be placed on the table and 13 revolutions be made in every ten seconds, it will be found quite easy to keep up a regular speed and get constant results. This regulation of speed is not so easy if one tries to count the 78 revolutions in the whole period of sixty seconds.
A similar calculation to the above in the albumen estimation tables (when a speed of 1,500 on the Purdy machine has been used) would necessitate a speed of 1,970 revolutions, or 16 turns of the handle in ten seconds. As the tubes supplied with the hand centrifuge are generally 10 cc. tubes (graduated to \( \frac{1}{10} \) of a c.c., except the two first c.c., which are graduated to twentieths), two-thirds of both the urine and the ingredients used by Purdy must be taken and the results obtained multiplied by \( \frac{3}{2} \). It is better, however, to obtain the well-graduated 15 c.c. tubes used by Purdy.

I have endeavoured to further extend the use of the centrifugal method by working out methods for estimating the urinary uric acid, total purins, purins apart from uric acid and the conjugated sulphates. The results so far obtained have been very encouraging, but the amount of labour required to work out the necessary tables and check the results is con-
siderable, and I have been unfortunately obliged to abandon the work—I hope temporarily—before its completion. I may say that the method devised for estimating the total purins is based on the Haycraft-Denigès' procedure described earlier in the paper. Instead, however, of combining the two parts 1 and 2 of solution A, described on page 326, I first add the ammonia-magnesia mixture to precipitate out the phosphates, and then add the \( \frac{x}{y} \) silver solution. The resulting precipitate of silver and purins is centrifugated, and its amount calculated and checked by control experiments with the Haycraft-Denigès' method.

The uric acid (alone) is calculated on a method allied to the Denigès' procedure for calculating this body: the phosphates are eliminated by solution 1 containing carbonate of soda (page 327), and uric acid precipitated out from the filtrate by the addition of a given quantity of Fehling's solution acted on by an alkaline bisulphate. Uric acid is alone precipitated out under these conditions, and can be centrifugated out as a urate of copper.

The total sulphates are estimated by treating the urine with strong hydrochloric acid in the presence of barium chloride and boiling for ten minutes, the resulting precipitate representing the total sulphates being centrifugated and measured. Purdy does not use heat when estimating the total sulphates by the centrifugal method, and cannot, as far as I am aware, completely precipitate out his conjugated sulphates.

The conjugated sulphates are estimated in a similar manner to the total sulphates in the filtrate of a urine to which the chloride of barium and a little acetic acid have been added to precipitate out the mineral sulphates.

The above methods of estimating the total and conjugated sulphates appear—so far as I have gone—to give results sufficiently accurate for relative determination of the salts. The method is certainly a rapid and simple one.

Conclusions.—If accurate centrifugal methods can be devised, as now seems probable, for estimating most of the urinary constituents, quantitative urinary analysis will become so simple a matter as to be undertaken in all hospitals of any size where a hand centrifuge exists and a few chemicals are procurable.

While quantitative urine work is confined to a few meta-
bolism experiments in selected laboratories we shall continue
to lose the great opportunities which we all have of finding out
something of the conditions of metabolic exchange and body
nutrition in various diseases; of organic resistance to disease,
and the effect of diseases on the nutritional rhythm. The
microbe has had considerably more attention paid to it than
has the chemical nature and cell life of its occasional medium
of growth—the human body.

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