A SYSTEM OF BLOOD ANALYSIS.

BY OTTO FOLIN AND HSIEN WU.

(From the Biochemical Laboratory, Harvard Medical School, Boston.)

(Received for publication, March 29, 1919.)

CONTENTS.

Introduction .......................................................... 81
Preparation of protein-free blood filtrates ..................................... 82
Determination of non-protein nitrogen .................................. 87
“ urea.......................................................... 91
“ creatinine and creatine...................................... 98
“ uric acid.................................................. 100
New Method for Determination of sugar................................. 106

INTRODUCTION.

The main purpose of the research recorded in this paper has been to combine a number of different analytical procedures into a compact system of blood analysis, the starting point for which should be a protein-free blood filtrate suitable for the largest possible number of different determinations. It need scarcely be pointed out what a convenience and advantage it would be if one could take the whole of a given sample of blood and at once prepare from it a protein-free blood filtrate suitable for the determination of all or nearly all the water-soluble constituents, non-protein nitrogen, urea, creatinine, creatine, uric acid, and sugar.

In connection with our work on the problem we have also had in mind the desirability of reducing as far as practicable the amount of blood filtrate to be used for each determination, for by means of such reduction the total usefulness of the filtrate is correspondingly increased. There is no hard and fast limit as to the extent to which this reduction can be carried. It is doubtful, however, whether it is sound analytical practice regularly to use the smallest possible amount of material for each determination; whether, for example, blood filtrates corresponding to only 0.1 cc.
Blood Analysis

of blood should regularly be used for non-protein nitrogen determinations, because it may sometimes be advantageous or necessary to take no more. In this paper we deal chiefly with a semi microchemical scale of work representing only a moderate reduction of the quantities ordinarily taken for colorimetric work with the 60 mm. Duboscq colorimeter.

One of the main obstacles encountered in attempts to develop a definite system of blood analysis of the kind we have had in mind has been the determination of the uric acid. For several years we have had serious doubts as to the full trustworthiness of the uric acid results heretofore recorded in the literature; moreover, to be reasonably accurate the determination has required more blood (about 25 cc.) than can be obtained except in isolated special cases. A large share of the work involved in this research has therefore been a critical study of the uric acid determination; and a modification of the Folin-Denis-Benedict method has been developed which requires the filtrate from only 2 cc. of blood, and which we believe to be more dependable as well as more simple and convenient than the original method.

We have also satisfactorily solved the problem of how to make and keep standard uric acid solutions, and we have devised a new colorimetric method for the determination of sugar in blood.

The determinations included in this research, namely non-protein nitrogen, urea, creatinine, creatine, uric acid, and sugar, can all be determined in the filtrate obtained from 10 cc. of blood.

Preparation of Protein-Free Blood Filtrates.

The pivotal point in our projected general scheme of blood analysis was necessarily a searching review of the most promising methods which have been used for precipitating the blood proteins in connection with the various analytical procedures in common use. As a working principle or guide in this search we have first of all required that the procedure employed must permit the quantitative recovery of at least 10 mg. of uric acid and creatinine when added to 100 cc. of sheep, beef, or chicken blood, and that the total non-protein nitrogen must certainly be no higher than the figures obtained from a corresponding trichloracetic acid filtrate representing a 10 per cent trichloracetic acid concentration (in
the diluted unfiltered blood mixture)—or a corresponding 1.5 per cent m-phosphoric acid filtrate.

While we are not dependent on the urease method for the determination of the urea, we have, nevertheless, deemed it imperative that the blood filtrate must also be of such a character as readily to permit the use of the urease method for the determination of this important constituent. We do not claim to have exhausted this line of inquiry, for it is a laborious process to determine the merits and shortcomings of any particular reagent in connection with such a comprehensive program. None of the precipitation procedures described in recent years is free from serious shortcomings. Kahlbaum's phosphotungstic acid or sodium phosphotungstate (prepared by ourselves) met our requirements when used under certain very definite conditions, and for a time we concentrated our efforts on the standardization of these reagents and on the adaptation of the various analytical procedures to the blood filtrates obtained from them.

In connection with our work on sodium phosphotungstate we have discovered a new protein precipitant which probably has never before been used in blood analysis. We refer to it as 'new protein precipitant because so far as we have been able to learn it has never before been used in that capacity. This substance is tungstic acid. Tungstic acid, like sodium phosphotungstate or phosphotungstic acid, must be used in a definite way, but the necessary conditions are not difficult to find. Less than 1 gm. is used for the precipitation of the proteins from 10 cc. of blood, yet the precipitation is more complete than that produced by 10 gm. of trichloroacetic acid, and the filtrate obtained gives no trouble in connection with any of the determinatives so far investigated. Neither creatinine nor uric acid is carried down by the precipitate within the conditions to be described. As much as 20 mg. of uric acid may be added to 100 cc. of blood without incurring any loss by absorption.

The blood protein precipitation obtained by the help of tungstic acid is interesting. The precipitation is completed within a few seconds. When the mixture is shaken hard, the sound is almost like that of shaken mercury and the hardest kind of shaking will not produce more than a trace of foam. The precipitate is very fine, yet does not go through good filter paper and does not stop
Blood Analysis

up the pores. The filtration is slow, but the total amount of filtrate obtained is nearly as large as that obtained with trichloroacetic acid. If the precipitated mixture is heated in a water bath for 2 or 3 minutes, the precipitate settles spontaneously. With this modification, centrifuging can be substituted for the filtration as the supernatant liquid is water-clear and contains no more nitrogen than the unheated filtrate. For the present we do not care to recommend this process except for quantities of blood so small that one cannot afford to filter. The statement of Folin and Denis that no precipitation involving the use of heat is permissible is probably erroneous as has been pointed out by Bock, although it is true for the m-phosphoric acid precipitation and probably for many others. Unless some compelling advantage is gained by the use of heat, precipitation in the cold does seem to be the safer process.

There are many other points of interest to be investigated in connection with tungstic acid as a precipitant, but as these have no direct bearing on the problem of this research, further consideration of them here is omitted. As a precipitant for blood proteins we believe that tungstic acid will prove more useful than any other reagent yet proposed.

The precipitation of the blood proteins by means of our new reagent is made in the following manner. Transfer a measured amount of blood into a flask having a capacity of fifteen to twenty times that of the volume taken. Dilute the blood with 7 volumes of water and mix. With an appropriate pipette add 1 volume of 10 per cent solution of sodium tungstate (Na₂WO₄·2H₂O) and mix. With another suitable pipette add to the contents in the flask (with shaking) 1 volume of 3/5 normal sulfuric acid. Close the mouth of the flask with a rubber stopper and give a few vigorous shakes. If the conditions are right hardly a single air bubble will form as a result of the shaking. Much oxalate or citrate interferes with the coagulation and later with the uric acid determination. 20 mg. of potassium oxalate is ample for 10 cc. of blood. Citrate, except in the minimum amount, is to be avoided. When a blood is properly coagulated, the color of the coagulum gradually changes from pink to dark brown. If this change does not occur, the coagulation is incomplete, due, in every case we have encountered, to too much oxalate or citrate. In such an
emergency the sample may be saved by adding 2 normal sulfuric acid drop by drop, shaking vigorously after each addition and allowing the mixture to stand for a few minutes before adding more, until the coagulation is complete. Pour the mixture on a filter large enough to hold the entire contents of the flask and cover with a watch-glass. If the filtration is begun by pouring the first few cc. of the mixture down the double portion of the filter paper and withholding the remainder till the whole filter has been wet, the filtrates are almost invariably as clear as water from the first drop. If a filtrate is not perfectly clear, the first 2 or 3 cc. may have to be returned to the funnel. (Filter papers of the following diameters will meet all ordinary needs: 11, 12½, 15, and 18½ cm.)

It will be noted that the precipitation of the blood proteins is not made in volumetric flasks. Our procedure is adapted to the full use of practically all of a given sample of blood, for by this system 7, 9, or 12 cc. can be utilized just as well as 5 or 10. For this work we have devised a special blood pipette,¹ a sketch of which is given in Fig. 1. This is simply a 15 cc. pipette, graduated from the long tip into 1 cc. portions. The lower part is more or less like that of a volumetric pipette, thus permitting one to draw the blood directly from small, narrow bottles. We find it convenient to use three such pipettes; one for the blood, one for the sodium tungstate solution, and one for the sulfuric acid. The water used for diluting the blood may be measured with a cylinder.

The preparation of protein-free blood filtrates by this new process is so simple that no one need go astray, provided that the sodium tungstate and the ²⁄₃ normal sulfuric acid are correct. The only doubtful point is the quality of the sodium tungstate used. The acid is intended to set free the whole of tungstic acid with about 10 per cent excess (and to neutralize the carbonate usually present in commercial tungstates). A

¹ The pipettes are made for us by the Emil Greiner Co., New York.
greater excess of sulfuric acid must not be used, for if this is the case a large part of the uric acid will be lost. A safe and convenient criterion is to test the blood filtrate obtained with Congo red paper. The reaction should be negative or at the most just perceptible. We have employed three different tungstates, and all worked equally well. The product we now use was obtained from the Primos Chemical Company, Primos, Pa.

The carbonate content of sodium tungstate is easily determined as follows: To 10 cc. of 10 per cent solution, add one drop of phenolphthalein and titrate with 0.1 normal hydrochloric acid. Each cc. of hydrochloric acid corresponds to 1.06 per cent of sodium carbonate. The amount of acid required for the titration should not exceed 0.4 cc.

Our blood filtrates are nearly neutral, 10 cc. of filtrate requiring only about 0.2 cc. of 0.1 normal sodium hydroxide when titrated with phenolphthalein as indicator. If the filtrates are to be kept for any length of time, more than 2 or 3 days, they need some preservation. One or two drops of toluene or xylene is adequate for the filtrate obtained from 10 cc. of blood. Xylene seems to be fully as effective as toluene as a preservative.

The precipitation process just described works equally well with any kind of blood which we have yet tried—human, beef, sheep,

### TABLE I.

Comparison of Non-Protein Nitrogen in Blood Filtrates Obtained by Means of Trichloroacetic Acid and Tungstic Acid.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trichloroacetic acid</th>
<th>Tungstic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>35.7</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
<td>32.4</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>35.4</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>50.7</td>
<td>48.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>68.5</td>
<td>54.5</td>
</tr>
<tr>
<td></td>
<td>48.0</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>53.2</td>
<td>44.4</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>51.6</td>
<td>47.6</td>
</tr>
</tbody>
</table>
chicken, dog, and rabbit—and numerous comparisons with the trichloroacetic acid precipitation have shown that the non-protein nitrogen obtained by the process invariably tends to be lower than the figures given by trichloroacetic acid. The figures of Table I illustrate this point.

Determination of Non-protein Nitrogen.

The protein-free blood filtrates prepared by our new process lend themselves perfectly to nitrogen determinations by the direct Nesslerization process of Folin and Denis. As a result of further experience with that method we are able to introduce certain modifications believed to represent improvements.

The acid digestion mixture is made as follows: Mix 300 cc. of phosphoric acid syrup (about 85 per cent H₃PO₄) with 100 cc. of concentrated sulfuric acid. Transfer to a tall cylinder, cover well to exclude the absorption of ammonia, and set aside for sedimentation of calcium sulfate. This sedimentation is very slow, but in the course of a week or so the top part is clear and 50 to 100 cc. can be removed by means of a pipette. (It is not absolutely necessary that the calcium should be thus removed, but it is probably a little safer to have it done.) To 100 cc. of the clear acid add 10 cc. of 6 per cent copper sulfate solution and 100 cc. of water. 2 cc. of this solution are substantially equivalent to 1 cc. of the acid mixture previously described by Folin and Denis. We prefer this diluted acid, first, because the objectionable viscosity of the undiluted reagent is practically eliminated, and, second, because we now use for a nitrogen determination only 5 cc. (instead of 10 cc.) of blood filtrate, and 1 cc. of acid (corresponding to 0.5 cc. of the undiluted acid reagent).

The micro-Kjeldahl digestion is made as before in test-tubes. While we still have an abundant supply of Jena test-tubes we no longer use them for this digestion because the Pyrex ignition test-tubes are very much better in nearly every respect. Test-tubes having a capacity of about 75 cc. (200 × 25 mm.) are suitable for this purpose, and if made of Pyrex ignition glass are almost as good as those of pure silica. These test-tubes should be graduated

Blood Analysis

at 35 cc. and at 50 cc. on two sides, or by means of diamond marks going entirely around. The reason for this graduation is that we now Nesslerize in the digestion tube.

In micro-Kjeldahl digestions severe bumping is much more common than in ordinary macro-Kjeldahl digestions, but even in the latter the bumping phenomenon is often a source of serious difficulties. Glass beads, pumice stone, pieces of porcelain, etc., are used to remedy this trouble. For years ordinary quartz pebbles have been used in this laboratory, but at times these too have failed to prevent loss of a determination through sudden violent bumping. Occasionally a pebble may be hurled out of a 200 mm. test-tube by one intensive explosion. At other times no trouble at all is encountered. Langstroth,⁵ who seems to have encountered very severe and persistent bumping, resorts to the device of holding the test-tube in as nearly a horizontal position as the contents in the tube will permit; but since it takes half an hour to boil off the liquid which ordinarily can be boiled off in less than 10 minutes, that remedy cannot be considered satisfactory. Langstroth seems to have concluded that such bumping is peculiar to blood filtrates obtained by means of m-phosphoric acid, but the phenomenon is quite general. Prolonged boiling of pure water in any glass vessel will lead to the most intense bumping. The most important cause of bumping is certainly the condition of the test-tube. A new test-tube does not cause bumping, but if the same one is used over and over again in one session, the bumping becomes progressively worse. The worst kind of a test-tube (or Kjeldahl flask) can be made as good as a new one by thoroughly drying it; also by rinsing with alcohol.

The reason why dry test-tubes cause less bumping and why dry pebbles tend to prevent bumping is manifestly the presence of very fine pores filled with air in the test-tube and in the pebbles. Until this air has been driven out by heat, localized formation of steam occurs and the boiling is smooth and even, but as these pores are gradually filled with the liquid the bumping begins. By keeping on hand a sufficiently large number of dry test-tubes so that no one need be used more than twice in one session the bumping phenomenon, in the presence of a fresh (that is a dry) quartz pebble or piece of granite, is almost entirely eliminated.

The Nesslerization process has also been simplified. The pre-
liminary neutralization of the acid has been eliminated. The
Nessler solution which we now use for all Nesslerizations is made
as follows: The stock solution of mercuric potassium iodide can
be made just as previously described. Dissolve 150 gm. of po-
tassium iodide in 100 cc. of warm water, add 200 gm. of mercuric
iodide, stir until the latter is dissolved, and dilute to a volume of
about 1 liter; filter, if necessary, and dilute to a final volume of 2
liters. It is advantageous to make a large volume of this solution
for a second sediment may form which takes a long time to settle.

The mercuric iodide obtainable from dealers frequently contains
insoluble impurities (probably mercuric sulfide and mercurous
iodide) which make it difficult to obtain a clear solution by the
addition of potassium iodide. In such cases it is advisable to let
the dissolved double iodide stand for 1 or 2 days and then filter,
before diluting to volume.

Because of the difficulties encountered in obtaining high grade
mercuric iodide, we have devised a new process for making the
mercuric potassium iodide solution. This process is as follows:
Transfer 150 gm. of potassium iodide and 110 gm. of iodine to a
500 cc. Florence flask; add 100 cc. of water and an excess of metal-
lic mercury, 140 to 150 gm. Shake the flask continuously and
vigorously for 7 to 15 minutes or until the dissolved iodine has
nearly disappeared. The solution becomes quite hot. When the
red iodine solution has begun to become visibly pale, though still
red, cool in running water and continue the shaking until the red-
dish color of the iodine has been replaced by the greenish color of
the double iodide. This whole operation usually does not take
more than 15 minutes. Now separate the solution from the sur-
plus mercury by decantation and washing with liberal quantities
of distilled water. Dilute the solution and washings to a volume
of 2 liters. If the cooling is begun in time, the resulting reagent
is clear enough for immediate dilution with 10 per cent alkali and
water, and the finished solution can at once be used for Nessler-
izations.

The cost of the chemicals called for in this process of making
Nessler's solution is less than when starting with mercuric iodide,
and the disagreeable impurities present in many samples of mer-
curic iodide are avoided.
From the stock solution of mercuric potassium iodide, made according to either of the processes described above, we prepare the final Nessler solution as follows: From completely saturated caustic soda solution containing about 55 gm. of NaOH per 100 cc. decant the clear supernatant liquid and dilute to a concentration of 10 per cent. (It is worth while to determine by titration that a 10 per cent solution has been obtained within an error of not over 5 per cent.) Introduce into a large bottle 3,500 cc. of 10 per cent sodium hydroxide solution, add 750 cc. of the double iodide solution and 750 cc. of distilled water, giving 5 liters of Nessler’s solution.

The Nessler solution so obtained contains enough alkali in 15 cc. to neutralize 1 cc. of the diluted phosphoric-sulfuric acid mixture and to give a suitable degree of alkalinity for the development of the color given by ammonia at a volume of 50 cc.

(In other Nesslerizations, as in urine analysis when there is no acid to be neutralized, 10 cc. of the Nessler reagent per 100 cc. of Nesslerized ammonia solution is the correct amount.)

**Concise Description of Non-Protein Nitrogen Determination.**—Introduce 5 cc. of the protein-free blood filtrate into a dry 75 cc. test-tube graduated at 35 cc. and at 50 cc. Add 1 cc. of the sulfuric-phosphoric acid mixture described on page 87. Add a dry quartz pebble and boil vigorously over a microburner until the characteristic dense acid fumes begin to fill the test-tube. This is usually accomplished in from 3 to 7 minutes. When the fumes are unmistakable, cut down the size of the flame so that the contents of the tube are just visibly boiling, and close the mouth of the test-tube with a watch-glass or a very small Erlenmeyer flask. Continue the heating very gently for 2 minutes from the time the fumes began to be unmistakable, even if the solution has become clear and colorless at the end of 20 to 40 seconds. If the oxidations are not visibly finished at the end of 2 minutes the heating must be continued until the solution is nearly colorless. Such cases are very rare; the oxidation is almost invariably finished within the 1st minute. Allow the contents to cool for 70 to 90 seconds and then add 15 to 25 cc. of water. Cool further, approximately to room temperature, and add water to the 35 cc. mark. Add, preferably with a pipette, 15 cc. of the Nessler solution described above. Insert a clean rubber stopper and mix. If the solution
is turbid, centrifuge a portion before making the color comparison with the standard. The standard most commonly required is 0.3 mg. of N (in the form of ammonium sulfate) in a 100 cc. flask. Add to it 2 cc. of the sulfuric-phosphoric acid mixture, about 50 cc. of water, and 30 cc. of Nessler solution. Fill to the mark and mix. The unknown and the standard should be Nesslerized at approximately the same time. If the standard is set at 20 mm. for the color comparison, 20 divided by the reading and multiplied by 30 gives the non-protein nitrogen in mg. per 100 cc. of blood.

**Determination of Urea.**

Investigations on the most satisfactory method for the determination of urea have been pursued for the last 2 or 3 years (partly with the assistance of G. L. Foster and Guy Youngburg). Much of the work done on the subject has been an endeavor to find a direct Nesslerization process without the use of Merck's blood charcoal. Our attempts have not resulted in any thoroughly satisfactory method because very small amounts of ammonia cannot be Nesslerized in the presence of either amino-acids or peptones. Direct Nesslerization, even with the help of charcoal, cannot be made except at the expenditure of more blood filtrate than is actually used in the final stages of the determination, and a strictly economical use of the blood filtrate we have considered a fundamentally important point in our system of blood analysis. Direct Nesslerization has therefore been abandoned in connection with the determination of urea in blood. Extensive use has also been made of the permutit extraction after first decomposing the urea with urease, but this process has proved somewhat fallacious with bloods in which the total urea nitrogen is small, as in many normal bloods, so this process also has been abandoned. Since probably no other determination will be as useful and important to the clinician as the determination of the blood urea, we have considered it of the utmost importance to get a method which is as simple as possible, but above all reliable. In this connection we have had in mind not only the needs of well equipped hospital laboratories, but also the needs of private practitioners.
For the hydrolysis of the urea we make use of jack bean urease, or the autoclave; for the isolation of the ammonia produced we employ aeration or distillation; thus we have four combinations any one of which will give satisfactory results. The autoclave process is, of course, not advantageous for single urea determinations, but on the other hand is distinctly useful when it is a question of a large series of determinations, or when creatine determinations are also to be made, because the hydrolysis of the urea can then be accomplished simultaneously with the conversion of the creatine into creatinine. The chief merit of the autoclave process for decomposing urea in blood filtrates lies perhaps in the fact that by its help one is sure to get all the urea nitrogen; the values obtained may be too high, but not too low. Yet the results obtained by the autoclave process are as a matter of fact usually identical and rarely as much as 1 mg. per 100 cc. of blood higher than those obtained by the urea process.

Urease Decomposition.—For the decomposition of urea by means of urease we use exclusively jack bean powder extracts and not so called purified or concentrated urease preparation. It is doubtful possible to prepare such, but those obtainable in the market are usually less active than an equal weight of jack bean powder, and of course are much more expensive. An excellent urease solution can be prepared from jack bean powder in the following manner: Transfer to a 200 cc. flask or bottle about 3 gm. of permutit powder. Wash this by decantation, once with 2 per cent acetic acid, then twice with water. Add to the moist permutit in the flask 100 cc. of 30 per cent alcohol (35 cc. of 95 per cent alcohol mixed with 70 cc. of water). Then introduce 5 gm. of jack bean meal and shake for 10 minutes. Filter and collect the filtrate in three or four different small clean bottles. Set one aside for immediate use; it will remain serviceable at least 1 week at ordinary room temperature, if not exposed to direct sunlight. Put the others on ice where they will remain good for 3 to 5 weeks. The filtrate contains substantially the whole of the urease present in the jack bean powder and is very active. In the presence of a suitable phosphate mixture, 1 cc. added to 300 mg. of urea nitrogen at a volume of 200 cc. will yield 37 to 42 mg. of urea nitrogen

4 The Arlington Chemical Co. supplies jack bean meal in a finer state of division than one can readily make by hand.
in 1 hour at 20°C. In 18 hours all the urea will be decomposed. The use of permutit makes the extract free from ammonia (5 cc. containing less than 0.01 mg.), nor does more ammonia develop on standing.

Urease decompositions of urea are never dependable except in the presence of some buffer mixtures by which the reaction of the solution can be kept within certain limits. The action of such mixtures is twofold. They not only accelerate the decomposition of the urea, but also prolong greatly the acting period of the enzyme. When urease solutions prepared as described above are added to urea dissolved in distilled water, it not infrequently happens that the enzyme acts for only a few minutes and then stops altogether, so that no more ammonia is obtained after 24 hours than after 15 minutes. That the enzyme is only dormant and not entirely destroyed is shown by the fact that on adding phosphate mixture to the solution after 24 or even 48 hours standing, renewed urea decomposition begins and then continues for a long time. The Auxourease found by Jacobi to be present in blood serum represents probably nothing more or less than a preserving action of amphoteric serum proteins on the urease, action similar to that of phosphates.

In the course of our investigations on the determination of urea in blood filtrates by means of urease, it was accidentally found that other phosphates than those investigated by Van Slyke are equally good or better. When the titratable acidity of m-phosphoric acid blood filtrates was neutralized with sodium bicarbonate, the urease action on (added) urea was surprisingly active and long sustained. (The urea content of such blood filtrates can be determined conveniently both by the urease and by the autoclave processes.) In consequence of this discovery, a series of experiments was made with pyro- and m-phosphates, and our observations have led to the conclusion that a solution containing 140 gm. of sodium pyrophosphate (u.s.r.) and 20 gm. of glacial phosphoric acid per liter is probably better than any of the phosphate mixtures investigated by Van Slyke. We are at a loss for an explanation, for Van Slyke's mixtures cover the field sufficiently well from the standpoint of hydrogen and hydroxyl ion concentrations.

Neumann, R., Über die Aktivierung der Soja-Urease durch menschliches Serum, Biochem. Z., 1915, lxix, 134.
A thorough study of this subject has not been made, but it appears that the pyrophosphates are less injurious to urease than o-phosphates. One experiment may be cited.

Solutions containing mono- and disodium phosphate in the molecular ratios 1:1 and 1:2 were prepared. To 300 mg. of urea nitrogen in 200 cc. flasks were added (1) 5 cc. of phosphate 1:1, (2) 5 cc. of phosphate 1:2, and (3) 5 cc. of the pyrophosphate solution described above. To such mixtures were added water to 200 cc. and 1 cc. of urease solution (temperature 18°C.). Table II shows the results.

**TABLE II.**
Comparison of Effect of Different Buffer Mixtures on Rate of Hydrolysis of Urea by Action of Urease.

<table>
<thead>
<tr>
<th>Buffer mixture</th>
<th>Ammonia N.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min.</td>
</tr>
<tr>
<td>Phosphate 1:1.</td>
<td>5.7</td>
</tr>
<tr>
<td>&quot; 1:2.........</td>
<td>5.7</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>12.5</td>
</tr>
</tbody>
</table>

**Determination of Urea by Urease Decomposition and Distillation.**

Transfer 5 cc. of the tungstic acid blood filtrate to a clean and dry Pyrex ignition tube (capacity about 75 cc.). The graduated Pyrex tubes recommended for the non-protein nitrogen determination should never be used for urea determinations, because they have contained Nessler solutions and Nessler solutions leave behind films of mercury compounds which destroy the urease. If those tubes must be used, they should first be washed with nitric acid to remove the mercury films. Add to the blood filtrate two drops of the pyrophosphate solution described above or two drops of a molecular o-phosphate solution (½ molecular monosodium phosphate plus ⅓ molecular disodium phosphate). Then add 0.5 to 1 cc. of the urease solution described on page 92 and immerse the test-tube in a beaker of warm water and leave it there for 5 minutes. The temperature of the water is not very important but should not exceed 55°C. The warm water can perhaps scarcely be said to be essential, for the hydrolysis is very rapid at room
temperature, but we nevertheless much prefer to use it. If no hot water is used, continue the digestion for 10 to 15 minutes, or as much longer as is convenient. The ammonia formed can be conveniently and quickly distilled into 2 cc. of 0.05 normal hydrochloric acid contained in a second test-tube. The second test-tube should not be so heavy as the ordinary test-tubes and should be graduated at 25 cc. A simple and compact arrangement for this distillation is indicated by Fig. 2. The test-tube which serves as a receiver is held in place by means of a rubber stopper in the side of which has been cut a fairly deep notch to permit the escape of air (and some steam). The rubber stopper serving as a holder for the receiver fits quite loosely to the delivery tube by means of which the two test-tubes are connected. The delivery tube must, of course, be so adjusted as to reach below the surface of the hydrochloric acid solution in the receiver before the distillation is begun.

Add to the hydrolyzed blood filtrate a dry pebble, 2 cc. of saturated borax solution, and a drop or two of paraffin oil; insert firmly the rubber-stopper carrying both delivery tube and receiver, and boil moderately fast over a microburner for 4 minutes. The size of the flame should never be cut down during the distillation, nor should the boiling be so brisk that the emission of steam from the receiving tube begins before the end of 3 minutes. At the end of 4 minutes slip off the receiver from the rubber stopper and put it in the position shown in Fig. 2. Continue the distillation for 1 more minute and rinse off the lower outside part of the delivery tube with a little water. Cool the distillate with running water, dilute to about 20 cc., and add 2.5 cc. of the Nessler solution described on page 90. Fill to the 25 cc. mark and compare in the colorimeter with a standard containing 0.3 mg. of N in a 100 cc. flask and Nesslerized with 10 cc. of the Nessler solution. The standard and unknown should always be Nesslerized as nearly simultaneously as practicable.

Calculation.—Multiply 20 (the height of the standard in mm.) by 15 and divide by the colorimetric reading to get the urea nitrogen per 100 cc. of blood. The reasons for this calculation are, of course, to be found in the fact that the standard containing 0.3

* The distillation apparatus can be obtained from Knott Apparatus Co., Boston.
Fig. 2. A, at beginning; B, toward end of distillation.
mg. of N is diluted to 100 cc., while the unknown, which corresponds to 0.5 cc. of blood, is diluted to only 25 cc.

It is even more important in this distillation than in the non-protein nitrogen digestion that the Pyrex test-tube should not be in a condition that leads to bumping. Dry the tube, or rinse it with alcohol, after each determination.

Borax, the alkali used in this distillation, is strong enough to set free the ammonia, yet is so weak that the blank ammonia which it gives with 5 cc. of urease solution is scarcely any greater than that obtained by the aeration process.

It will be noted that no condenser is used in connection with the microdistillation described above. Since ammonia can be quantitatively recovered by means of an air current, it would seem that it should be recovered as easily by means of a current of steam, especially since the first part of the distillate, containing probably 90 per cent of the ammonia, is automatically condensed just as in ordinary macro-Kjeldahl distillations. A few experiments made along this line indicate that condensers are indeed superfluous even in macro-Kjeldahl distillations.

The other three modifications for the determination of urea in the blood filtrates can be referred to very briefly, for they will be used only by those who are already familiar with the principles and practices involved.

**Urea Determination by Means of Urease and Aeration.**—The decomposition of the urea is made in the same kind of a Pyrex test-tube and in the manner already described. 1 or 2 cc. of 10 per cent sodium hydroxide are added and the ammonia is aspirated into a test-tube graduated at 25 cc. and containing 2 cc. of 0.05 normal hydrochloric acid. The only precaution which experienced investigators are likely to overlook is that the rubber tubing used for connections needs to be rinsed with water before being used the first time, and, later also, if the tubing has been idle for any length of time. The talcum powder with which the inner and outer surface of rubber tubing is coated is probably the source of the trouble in the case of new rubber tubing. It is probably contaminated with ammonia.

**Urea Determination by Means of Autoclave Decomposition.**—To 5 cc. of blood filtrate in a 75 cc. test-tube is added 1 cc. of normal acid; the mouth of the test-tube is covered with tin-foil, and the
Blood Analysis

test-tube with contents is then heated in the autoclave at 150°C. for 10 minutes.

Allow the autoclave to cool to below 100°C. before opening. The ammonia is then distilled off exactly as in the first process described except that 2 cc. of 10 per cent sodium carbonate are substituted for the borax or it is removed by aeration in the usual manner. The autoclave process is of course only an adaptation of the process first recommended and then abandoned by Benedict for the determination of urea in urine. We are not prepared to say that in terms of per cent the results may not be as much too high in our blood filtrates as they were found to be in urine. An error of several per cent is, however, not at all important in the determination of the urea in blood. Whether one finds 15 instead of 14 mg. of urea nitrogen in human blood, or whether one obtains 3 instead of 2 mg. in 100 cc. of chicken blood is as yet of comparatively small consequence.

**Determination of Creatinine and Creatine.**

In this section we shall describe fairly obvious applications to our blood filtrate of Folin's colorimetric method for the determination of creatinine and creatine without thereby implying that the results so obtained are more accurate than the results which can be obtained by various other modifications which have been proposed during the past 2 or 3 years. The original methods as applied to blood were devised for the purpose of studying the absorption of creatinine and creatine and were adequate for that problem. Subsequent experience in many laboratories has shown that the method for the creatine gives results that are too high. The false step introduced in connection with the creatine determinations was undoubtedly the employment of picric acid as a protein precipitant, although at the time this seemed a peculiarly suitable process for securing the creatinine in concentrations then deemed necessary for reliable color comparisons. The process could perhaps be saved if it were worth while, for the cause of the high results is probably the formation of traces of hydrogen sulfide during the heating in the autoclave. The method is, however, now superfluous.

**Determination of Preformed Creatinine.**—Transfer 25 (or 50) cc. of a saturated solution of purified picric acid to a small, clean flask,
add 5 (or 10) cc. of 10 per cent sodium hydroxide, and mix. Transfer 10 cc. of blood filtrate to a small flask or to a test-tube, transfer 5 cc. of the standard creatinine solution described below to another flask, and dilute the standard to 20 cc. Then add 5 cc. of the freshly prepared alkaline picrate solution to the blood filtrate, and 10 cc. to the diluted creatinine solution. Let stand for 8 to 10 minutes and make the color comparison in the usual manner, never omitting first to ascertain that the two fields of the colorimeter are equal when both cups contain the standard creatinine picrate solution. The color comparison should be completed within 15 minutes from the time the alkaline picrate was added; it is therefore never advisable to work with more than three to five blood filtrates at a time.

When the amount of blood filtrate available for the creatinine determination is too small to permit repetition, it is of course advantageous or necessary to start with more than one standard. If a high creatinine should be encountered unexpectedly without several standards ready, the determination can be saved by diluting the unknown with an appropriate amount of the alkaline picrate solution—using for such dilution a picrate solution first diluted with two volumes of water—so as to preserve equality between the standard and the unknown in relation to the concentration of picric acid and sodium hydroxide.

One standard creatinine solution, suitable both for creatinine and for creatine determinations in blood, can be made as follows: Transfer to a liter flask 6 cc. of the standard creatinine solution used for urine analysis (which contains 6 mg. of creatinine); add 10 cc. of normal hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add four or five drops of toluene or xylene. 5 cc. of this solution contain 0.03 mg. of creatinine, and this amount plus 15 cc. of water represents the standard needed for the vast majority of human bloods, for it covers the range of 1 to 2 mg. per 100 cc. In the case of unusual bloods representing retention of creatinine, take 10 cc. of the standard plus 10 cc. of water, which covers the range of 2 to 4 mg. of creatinine per 100 cc. of blood; or 15 cc. of the standard plus 5 cc. of water by which 4 to 6 mg. can be estimated. By taking the full 20 cc. volume from the standard solution at least 8 mg. can be estimated; but when working with such blood it is well to consider whether
it may not be more advantageous to substitute 5 cc. of blood filtrate plus 5 cc. of water for the usual 10 cc. of blood filtrate.

Calculation.—The reading of the standard in mm. (usually 20) multiplied by 1.5, 3, 4.5, or 6 (according to how much of the standard solution was taken), and divided by the reading of the unknown, in mm., gives the amount of creatinine, in mg. per 100 cc. of blood. In connection with this calculation it is to be noted that the standard is made up to twice the volume of the unknown, so that each 5 cc. of the standard creatinine solution, while containing 0.03 mg., corresponds to 0.015 mg. in the blood filtrate.

Determination of Creatine plus Creatinine.—Transfer 5 cc. of blood filtrate to a test-tube graduated at 25 cc. These test-tubes are also used for urea and for sugar determinations. Add 1 cc of normal hydrochloric acid. Cover the mouth of the test-tube with tin-foil and heat in the autoclave to 130°C. for 20 minutes or, as for the urea hydrolysis, to 155°C. for 10 minutes. Cool. Add 5 cc. of the alkaline picrate solution and let stand for 8 to 10 minutes, then dilute to 25 cc. The standard solution required is 20 cc. of creatinine solution in a 50 cc. volumetric flask. Add 2 cc. of normal acid and 10 cc. of the alkaline picrate solution and after 10 minutes standing dilute to 50 cc. The preparation of the standard must of course have been made first so that it is ready for use when the unknown is ready for the color comparison. The height of the standard, usually 20 mm., divided by the reading of the unknown and multiplied by 6 gives the "total creatinine" in mg. per 100 cc. blood.

In the case of uremic bloods containing large amounts of creatinine 1, 2, or 3 cc. of blood filtrate, plus water enough to make approximately 5 cc., are substitutes for 5 cc. of the undiluted filtrate.

The normal value for "total creatinine" given by this method is about 6 mg. per 100 cc. of blood.

Determination of Uric Acid.

The colorimetric method for the determination of uric acid in blood, like the colorimetric method for the determination of creatinine in urine, has furnished a tangible starting point for much important research. With the introduction by Benedict of po-
taosium cyanide (or, as we prefer, sodium cyanide) for dissolving the silver urate, the uric acid method was materially simplified, and a new impetus was given to a widespread use of the process in researches of various kinds. For about 3 years doubts have been strong in this laboratory as to whether the method is really as reliable as it was at first believed to be, and in this laboratory at least we decided not to make further applications of it in research until these doubts could be removed. Our misgivings have proved in part unfounded and in part correct. Our fear that relatively large traces of uric acid are carried down with the blood proteins, during the coagulation process, have proved substantially groundless. On the other hand, it is certainly true that the precipitation of the uric acid from the concentrated blood filtrates by means of magnesia mixture and silver lactate (i.e., essentially by Salkowski's process) is not quantitative, and the solubility of the silver urate is so large as to involve serious errors. On precipitating 0.1 mg. of uric acid from 10 cc. of solution an average loss of 50 per cent is encountered. By taking sufficiently large quantities of blood, 25 cc., the error due to the solubility of silver urate is of course largely eliminated, but the practical usefulness of the process is thereby much diminished. Another variable and uncontrollable source of error in the method is encountered during the concentration of the blood filtrates. If the total amount of water (acidified with acetic acid) to be boiled off does not exceed 100 cc. there is usually no destruction of uric acid, but when the volume is 200 to 400 cc. the losses, though variable, frequently amount to from 10 to 20 per cent, when starting with 0.1 or 0.2 mg. of uric acid. This source of error also can be eliminated almost wholly by taking aliquot portions of the blood filtrate (instead of the whole plus wash water). As a control method we have found the following process useful.

Heat about 160 cc. of water to boiling in a previously weighed beaker (capacity 500 cc.). Add 2 cc. of normal acetic acid, and add with pipettes 40 cc. of blood. Heat with constant stirring until the mixture is again boiling and continue the boiling for 2 minutes. Transfer beaker and contents to the scales, and add water until the total weight of the contents amounts to 200 gm. Mix and filter immediately. Transfer 100 cc. of the water-clear filtrate to an evaporating dish, add 1 cc. of 25 per cent acetic acid,
and boil down as rapidly as possible to a volume of about 5 cc. Transfer the residue to a 15 cc. centrifuge tube, rinsing with 1 to 2 cc. of 0.1 per cent lithium carbonate solution. Cool. Add 2 cc. of Benedict's ammoniacal silver magnesia mixture, stir for 2 minutes, and centrifuge. Decant as completely as possible the supernatant liquid. To the residue in the tube add 2 cc. of 5 per cent sodium cyanide solution; stir, add 10 to 13 cc. of water, stir, and centrifuge again. Transfer the supernatant liquid to a 100 cc. volumetric flask, and make the color comparison in the usual manner.

As a control method the process outlined above is good, and if we were dependent on the method for regular use we should cut it down and introduce corrections for the solubility of silver urate.

Before describing the determination of uric acid in tungstic acid blood filtrates we wish to describe the preparation of a new standard solution of uric acid—a solution the keeping quality of which we now, after 18 months of constant use, consider much superior to any other as yet devised. The solvent is 10 per cent sodium sulfite, and the keeping quality of the solution depends on the fact that the sulfite keeps the solution free from dissolved oxygen. The solution is prepared as follows:

Make 1 to 3 liters of a 20 per cent solution of sodium sulfite, let stand over night, and filter. Dissolve 1 gm. of uric acid in 125 to 150 cc. of 0.4 per cent lithium carbonate solution and dilute to a volume of 500 cc. Transfer 50 cc., corresponding to 100 mg. of uric acid, to each of a series of volumetric liter flasks. Add 200 to 300 cc. of water, then 500 cc. of filtered 20 per cent sodium sulfite solution, and finally make up to volume, and mix well. Fill a series of 200 cc. bottles, and stopper very tightly with rubber stoppers. The solution in a bottle which is opened daily will keep for at least 3 to 4 months. Our records kept for one larger bottle so used show that no measurable loss of uric acid had occurred at the end of 6 months. In unopened bottles we expect the uric acid to keep for many years.

The surplus 20 per cent sulfite solution should be diluted to concentration of 10 per cent and should then be transferred to another series of small, tightly stoppered bottles. This sulfite is added to the unknown in order to offset the sulfite content of the standard.
Solutions Required for Uric Acid Determinations.

1. The standard uric acid sulfate solution already described (3 cc. used for each series of determinations).
2. A 10 per cent sodium sulfate solution, also described (2 cc. used for each determination).
3. A 5 per cent sodium cyanide solution, to be added from a burette (2.5 to 5 cc. used for each series of determinations).
4. A 10 per cent solution of sodium chloride in 0.1 normal hydrochloric acid (10 to 20 cc. used for each series of determinations).
5. The uric acid reagent prepared according to Folin and Denis. A still stronger reagent is obtained by heating the sodium tungstate (100 gm.) and the phosphoric acid (80 cc.) plus water (700 cc.) for 24 hours, instead of 2 hours; but the advantage gained, about 20 per cent, is not needed. Dilute the solution to 1 liter.
6. A solution of 5 per cent silver lactate in 5 per cent lactic acid (4 to 5 cc. needed for each determination).

In our new method for the determination of uric acid the latter is precipitated directly from the filtrate, without any previous concentration. 20 cc. of filtrate corresponding to 2 cc. of blood are used. In describing the process we shall have to introduce a slight variation from the way we actually do it. This variation is due to the fact that we use a larger centrifuge than most laboratories possess and by means of which we are able to use 30 cc. test-tubes for the precipitation. Using the small 15 cc. centrifuge tubes, it is necessary either to precipitate 10 cc. of filtrate in each of two tubes or to make the precipitation in two 10 cc. installments.

To 10 cc. of blood filtrate in each of two centrifuge tubes add 2 cc. of a 5 per cent solution of silver lactate in 5 per cent lactic acid, and stir with a very fine glass rod. Centrifuge; add a drop of silver lactate to the supernatant solution, which should be almost perfectly clear and should not become turbid when the last drop of silver solution is added. Remove the supernatant liquid by decantation as completely as possible. Add to each tube 1 cc. of a solution of 10 per cent sodium chloride in 0.1 normal hydrochloric acid and stir thoroughly with the glass rod. Then add 5 to 6 cc. of water, stir again, and centrifuge once more. By this chloride treatment the uric acid is set free from the precipitate. Transfer the two supernatant liquids by decantation to a 25 cc. volu-
metric flask. Add 1 cc. of a 10 per cent solution of sodium sulfite, 0.5 cc. of a 5 per cent solution of sodium cyanide, and 3 cc. of a 20 per cent solution of sodium carbonate. Prepare simultaneously two standard uric acid solutions as follows:

Transfer to one 50 cc. volumetric flask 1 cc. and to another 50 cc. flask 2 cc. of the standard uric acid sulfite solution described above. To the first flask add also 1 cc. of 10 per cent sodium sulfite solution. Then add to each flask 4 cc. of the acidified sodium chloride solution, 1 cc. of the sodium cyanide solution, and 6 cc. of the sodium carbonate solution. Dilute with water to about 45 cc. When the two standard solutions and the unknown have been prepared as described they are ready for the addition of the uric acid reagent of Folin and Denis. Add 0.5 cc. of this reagent to the unknown and 1 cc. to each of the standards, and mix. Let stand for 10 minutes, fill to the mark with water, mix, and make the color comparison.

Calculation. In connection with the calculation it is to be noted (a) that the blood filtrate taken corresponds to 2 cc. of blood, (b) that the standard is diluted to twice the volume of the unknown, and (c) that the standard used contains 0.1 or 0.2 mg. of uric acid. The blood filtrate from blood containing 2.5 mg. of uric acid will be just equal in color to the weaker standard. 20 times 2.5 divided by the reading of the unknown gives, therefore, the uric acid content of the blood when the weaker standard is set at 20 mm.

The two standards recommended were adopted on the basis of the experience gained from the analysis of more than 150 different samples of human blood. About one-third of these bloods was from soldiers and most of the others were obtained from the State Wassermann Laboratory through the courtesy of Dr. Hinton. The bloods unfortunately do not cover the wider range occurring among hospital patients. A moderate number of blood samples have been obtained from the Massachusetts General Hospital, and these reveal that the uric acid may sink to as low as 1 mg. of uric acid per 100 cc. of blood. It seems hardly worth while to prepare a third and weaker standard regularly in order to provide for such low uric acid values. A standard corresponding to the color obtained from 1.25 mg. of uric acid per 100 cc. of blood can be prepared within a couple of minutes as follows:
Transfer 1 cc. of 10 per cent sulfite solution, 3 cc. of 20 per cent sodium carbonate, 2 cc. of the acidified sodium chloride, 0.5 cc. of the sodium cyanide solution, and 25 cc. of the weaker one of the two regular standard solutions already on hand. Dilute to 50 cc. and mix. Or, simply add 5 cc. of 20 per cent sodium carbonate to 25 cc. of the regular weaker standard, and dilute to 50 cc.

If a low uric acid value is expected, an alternate procedure is to dilute the unknown to a final volume of 10 cc. with corresponding reduction in the amount of the reagents used.

Special attention should perhaps be called to one small yet essential variation in the process for developing the blue uric acid color, a variation made necessary by the use of sodium sulfite. The uric acid reagent must invariably be added after, and not before, the addition of the sodium carbonate, because in acid solution the sulfite will itself give a blue color with the phosphotungstic acid.

It may also be worth while to mention that the peculiar increase in blue color obtained by the use of cyanide is not obtained in the presence of sulfite. Opinions will doubtless differ as to whether this is an advantage or disadvantage. The amount of color obtained from 2 cc. of blood is rather weak, and if we could conveniently have retained the intensifying effects of the cyanide we probably should have done so, though the fainter solutions can be

---

**TABLE III.**

Comparison of Old and New Methods For Determination of Blood Uric Acid.

<table>
<thead>
<tr>
<th>Source</th>
<th>New method.</th>
<th>Mg. per 100 cc. blood.</th>
<th>Old method.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without solubility correction.</td>
<td>With solubility correction.</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>2.6</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>3.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>2.8</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>3.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* Slightly modified.

---

O. Folin and H. Wu 105
Blood Analysis

read just as readily and accurately as the stronger ones obtained by means of the cyanide. The antifading effects of the cyanide are retained.

New Method for Determination of Sugar.

It was originally our intention to incorporate some adaptation of Benedict's picrate method for the determination of sugar to our tungstic acid blood filtrates. But a few exploratory experiments showed that an intense and stable color reaction can be obtained by the application of the phenol reagent of Folin and Denis to cuprous oxide. The color obtained from a given quantity of sugar is far more intense than that obtained by the alkaline picrate reaction; so that a small fraction of a mg. of dextrose (1 or 2 cc. of blood filtrate) is all that is required for a determination of the blood sugar. Some difficulties were encountered in trying to find the conditions under which the extent of reduction is strictly proportionate to the quantities of sugar used; but, by a systematic study of the various factors involved, these difficulties were overcome and a rapid and convenient process was obtained.

The copper solution used for reduction is a weakly alkaline copper tartrate solution. Qualitatively this solution is an extremely sensitive reagent for traces of sugar, yet is not affected by creatinine or uric acid in quantities corresponding to 50 mg. of each per 100 cc. of blood. We are therefore inclined to regard our method as more accurate than any method as yet proposed for the determination of sugar in blood.

The picrate methods,7 whether we use Benedict's last modification or Myers' modification of Benedict's original method, in our hands give almost invariably results that are materially higher than the figures given by our new method. We are under the impression that the picrate methods are subject to sources of error similar to those encountered in Folin's original picrate method for blood creatine. The development of color in blood filtrates seems

not to proceed at the same rate of speed as the color derived from
a corresponding amount of dextrose. If the heating is interrupted
at the end of 2 to 3 minutes the value obtained for the blood sugar
will be nearly 50 per cent higher than when the heating is continu-
ued for 10 minutes or more. Such quantitative variations are
not encountered in our process when equal amounts of dextrose
in the form of pure sugar and of blood filtrate are heated, except
that the reduced copper is, of course, more extensively precipitated
and visible in the pure sugar solution. It need scarcely be stated
that added sugar is quantitatively recovered by our method.

Solutions Needed for Determination of Sugar in Blood.

1. Standard Sugar Solution.—Dissolve 1 gm. of pure anhydrous
dextrose in water and dilute to a volume of 100 cc. Mix, add a
few drops of xylene or toluene, and bottle. If pure dextrose is
not available, a standard solution of invert sugar made from cane
sugar is equally useful. Transfer exactly 1 gm. of cane sugar to a
100 cc. volumetric flask; add 20 cc. of normal hydrochloric acid and
let the mixture stand over night at room temperature (or rotate the
flask and contents continuously for 10 minutes in a water bath kept
at 70°C.). Add 1.68 gm. of sodium bicarbonate and about 0.2
gm. of sodium acetate, to neutralize the hydrochloric acid. Shake
a few minutes to remove most of the carbonic acid and fill to the
100 cc. mark with water. Then add 5 cc. more of water (1 gm. of
cane sugar yields 1.05 gm. of invert sugar) and mix. Transfer to
a bottle; add a few drops of xylene or toluene, shake well, and
stopper tightly. The stock solution made in either way keeps
indefinitely. Dilute 5 cc. to 500 cc., giving a solution 10 cc. of
which contain 1 mg. of dextrose or invert sugar. Add some xylene.
Use 2 cc. for each determination.

2. Alkaline Copper Solution.—Dissolve 40 gm. of anhydrous
sodium carbonate in about 400 cc. of water and transfer to a liter
flask. Add 7.5 gm. of tartaric acid and when the latter has dis-
solved add 4.5 gm. of crystallized copper sulfate; mix, and make
up to a volume of 1 liter. If the carbonate used is impure, a sedi-
ment may be formed in the course of a week or so. If this hap-
pens, decant the clear solution into another bottle.
3. Phosphotungstic-phosphomolybdic Acid.—Transfer to a large flask 25 gm. of molybdenum trioxide (MoO₃) or 34 gm. of ammonium molybdate (NH₄)₂MoO₄; add 140 cc. of 10 per cent sodium hydroxide and about 150 cc. of water. Boil for 20 minutes to drive off the ammonia (molybdc acid sometimes contains large amounts of ammonia as impurity). Add to the solution 100 gm. of sodium tungstate, 50 cc. of 85 per cent phosphoric acid, and 100 cc. of concentrated hydrochloric acid. Dilute to a volume of 700 to 800 cc.; close the mouth of the flask with a funnel and watch-glass. Boil gently for not less than 4 hours, adding hot water from time to time to replace that lost during the boiling. Cool and dilute to 1 liter. This solution is identical with the phenol reagent of Folin and Denis. For use in connection with the determination of blood sugar dilute 1 volume (100 cc.) of the reagent with one-half volume (50 cc.) of water and one-half volume (50 cc.) of concentrated hydrochloric acid.

4. Saturated Sodium Carbonate Solution.

The determination of blood sugar is carried out as follows: Heat a beaker of water to vigorous boiling. Transfer 2 cc. of the tungstic acid blood filtrate to a test-tube (20 m. x 200 mm.) graduated at 25 cc. The graduated test-tubes used as receivers when distilling off the ammonia in urea determinations (p. 95) are suitable for this work. Transfer 2 cc. of the dilute standard sugar solution to another similar test-tube. Add to each tube 2 cc. of the alkaline copper tartrate solution. Heat in the boiling water for 6 minutes. Remove the test-tube and add at once (without cooling), preferably from a graduated pipette, 1 cc. of the strongly acidified and diluted phenol reagent. This should be done as nearly simultaneously as possible; it is not advisable to use one standard for a set of more than four determinations. The purpose of the added hydrochloric acid in the reagent is to dissolve the cuprous oxide. Mix, cool, and add 5 cc. of saturated sodium carbonate solution. An intense blue color is gradually developed which will remain unaltered for several days. Dilute the contents of both test-tubes to the 25 cc. mark, and after at least 5 minutes make the color comparison in the usual manner.

The depth of the standard (in mm.) multiplied by 100 and divided by the reading of the unknown gives the sugar content, in mg., per 100 cc. of blood.
TABLE IV.
Sample Analyses of Protein-Free Blood Filtrates Obtained by Means of Tungstic Acid.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>10</td>
<td>1.3</td>
<td>1.5</td>
<td>6.0</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>13</td>
<td>1.0</td>
<td>1.4</td>
<td>5.3</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>12</td>
<td>1.1</td>
<td>1.2</td>
<td>6.7</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>12</td>
<td>2.2</td>
<td>2.0</td>
<td>5.7</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>13</td>
<td>3.3</td>
<td>1.5</td>
<td>6.0</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>11</td>
<td>2.6</td>
<td>1.4</td>
<td>5.2</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>13</td>
<td>1.6</td>
<td>1.4</td>
<td>6.0</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>13</td>
<td>2.4</td>
<td>1.6</td>
<td>5.5</td>
<td>82</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>14</td>
<td>4.1</td>
<td>1.7</td>
<td>5.8</td>
<td>83</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>15</td>
<td>2.8</td>
<td>1.6</td>
<td>5.4</td>
<td>91</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>15</td>
<td>3.4</td>
<td>1.4</td>
<td>5.3</td>
<td>97</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>13</td>
<td>2.4</td>
<td>1.7</td>
<td>6.0</td>
<td>104</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>17</td>
<td>2.0</td>
<td>1.3</td>
<td>4.8</td>
<td>83</td>
</tr>
<tr>
<td>14</td>
<td>33</td>
<td>16</td>
<td>2.5</td>
<td>1.6</td>
<td>5.7</td>
<td>105</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>15</td>
<td>1.1</td>
<td>1.6</td>
<td>5.5</td>
<td>95</td>
</tr>
<tr>
<td>16</td>
<td>34</td>
<td>16</td>
<td>0.8</td>
<td>1.3</td>
<td>6.1</td>
<td>119</td>
</tr>
<tr>
<td>17</td>
<td>34</td>
<td>16</td>
<td>2.6</td>
<td>1.5</td>
<td>5.9</td>
<td>106</td>
</tr>
<tr>
<td>18</td>
<td>35</td>
<td>17</td>
<td>2.1</td>
<td>1.6</td>
<td>6.0</td>
<td>89</td>
</tr>
<tr>
<td>19</td>
<td>35</td>
<td>17</td>
<td>2.0</td>
<td>1.4</td>
<td>5.5</td>
<td>77</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>18</td>
<td>2.0</td>
<td>1.7</td>
<td>5.7</td>
<td>86</td>
</tr>
<tr>
<td>21</td>
<td>35</td>
<td>18</td>
<td>2.9</td>
<td>1.6</td>
<td>5.8</td>
<td>95</td>
</tr>
<tr>
<td>22</td>
<td>35</td>
<td>17</td>
<td>3.2</td>
<td>1.4</td>
<td>5.5</td>
<td>94</td>
</tr>
<tr>
<td>23</td>
<td>35</td>
<td>18</td>
<td>2.5</td>
<td>1.5</td>
<td>6.0</td>
<td>89</td>
</tr>
<tr>
<td>24</td>
<td>35</td>
<td>19</td>
<td>2.2</td>
<td>1.5</td>
<td>5.3</td>
<td>91</td>
</tr>
<tr>
<td>25</td>
<td>35</td>
<td>22</td>
<td>3.5</td>
<td>1.4</td>
<td>5.7</td>
<td>87</td>
</tr>
<tr>
<td>26</td>
<td>35</td>
<td>17</td>
<td>2.3</td>
<td>1.7</td>
<td>6.7</td>
<td>83</td>
</tr>
<tr>
<td>27</td>
<td>35</td>
<td>18</td>
<td>1.6</td>
<td>1.3</td>
<td>6.5</td>
<td>104</td>
</tr>
<tr>
<td>28</td>
<td>36</td>
<td>17</td>
<td>2.8</td>
<td>1.5</td>
<td>5.2</td>
<td>100</td>
</tr>
<tr>
<td>29</td>
<td>37</td>
<td>18</td>
<td>2.1</td>
<td>1.5</td>
<td>5.5</td>
<td>94</td>
</tr>
<tr>
<td>30</td>
<td>38</td>
<td>18</td>
<td>2.2</td>
<td>1.7</td>
<td>5.4</td>
<td>95</td>
</tr>
<tr>
<td>31</td>
<td>39</td>
<td>18</td>
<td>2.6</td>
<td>1.8</td>
<td>6.7</td>
<td>103</td>
</tr>
<tr>
<td>32</td>
<td>39</td>
<td>18</td>
<td>2.9</td>
<td>1.5</td>
<td>6.0</td>
<td>87</td>
</tr>
<tr>
<td>33</td>
<td>40</td>
<td>18</td>
<td>2.0</td>
<td>1.6</td>
<td>6.0</td>
<td>98</td>
</tr>
<tr>
<td>34</td>
<td>40</td>
<td>20</td>
<td>2.6</td>
<td>1.7</td>
<td>5.6</td>
<td>95</td>
</tr>
<tr>
<td>35</td>
<td>41</td>
<td>19</td>
<td>4.8</td>
<td>1.5</td>
<td>5.9</td>
<td>93</td>
</tr>
<tr>
<td>36</td>
<td>41</td>
<td>19</td>
<td>4.2</td>
<td>2.5</td>
<td>6.6</td>
<td>109</td>
</tr>
<tr>
<td>37</td>
<td>43</td>
<td>19</td>
<td>2.2</td>
<td>1.7</td>
<td>6.3</td>
<td>78</td>
</tr>
<tr>
<td>38</td>
<td>139</td>
<td>106</td>
<td>5.4</td>
<td>12.5</td>
<td>19.4</td>
<td>99</td>
</tr>
<tr>
<td>39</td>
<td>147</td>
<td>115</td>
<td>8.9</td>
<td>11.0</td>
<td>20.5</td>
<td>170</td>
</tr>
<tr>
<td>40</td>
<td>275</td>
<td>237</td>
<td>14.3</td>
<td>13.6</td>
<td>27.2</td>
<td>157</td>
</tr>
</tbody>
</table>
The copper solution is adjusted to give proportionate reductions with 0.12 to 0.4 mg. of dextrose. This covers the range of hypoglycemic and hyperglycemic bloods. But in extreme cases it is better to use 3 or 1 cc. of the filtrate, instead of 2 cc., adding water to the standard or to the unknown so as to equalize the concentration of the alkaline copper.
A SYSTEM OF BLOOD ANALYSIS
Otto Folin and Hsien Wu


Access the most updated version of this article at http://www.jbc.org/content/38/1/81.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/38/1/81.citation.full.html
#ref-list-1