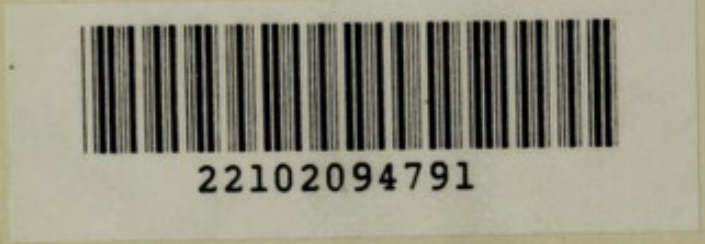
Blood chemistry, colorimetric methods for the general practitioner : with clinical comments and dietary suggestions / by Willard J. Stone.

BLOOD CHEMISTRY
COLORIMETRIC METHODS

EDWARD BARTLE SHAW



For Review.

BLOOD CHEMISTRY
COLORIMETRIC METHODS

BLOOD CHEMISTRY
COLORIMETRIC METHODS
For the General Practitioner

WITH CLINICAL COMMENTS
AND DIETARY SUGGESTIONS

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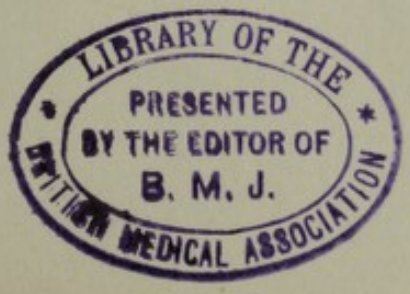

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PREFACE TO THE SECOND EDITION

The text has been rewritten in the hope that it may continue to stimulate interest in methods proved by experience to be readily available to every internist in his own laboratory. The interpretation of findings and their bearing upon the problems of clinical medicine will continue to be the most important feature of such work. Additions have therefore been made to the Clinical Comments from the rapidly growing literature of the subject, and the references consulted have been added. My thanks are due my secretary, Mildred P. Swanson, for her painstaking assistance, and Mr. Paul B. Hoeber, the publisher, for his many courtesies. I am also grateful to many friends who have offered criticisms.

W. J. S.

Pasadena, California
December, 1925.

PREFACE TO THE FIRST EDITION

It is hoped that those interested in the chemistry of the more common blood constituents affected by impaired function or disease will find this compilation useful because of the arrangement of the methods. The methods, which have been slightly modified to meet the demands of the clinical laboratory using relatively small quantities of blood, are dependent entirely upon the exhaustive researches of Folin and Wu, Denis, Lewis, Benedict, Van Slyke, Bloor, Myers, Bailey and others. Certain clinical comments have been added. The steps to be followed in the determination of total nitrogen in the urine have been modified from the original method of Folin in order to conform to the same strength nitrogen standard used in the determination of non-protein nitrogen in the blood. It has been added for the convenience of those who may be interested in the comparison of blood nitrogen retention, of output nitrogen in the urine and the intake nitrogen from weighed diets. Folin's method for the determination of urinary titratable acidity has been added. For the purpose of ready reference an outline of the essential facts to be determined in the study of impaired kidney function has also been included, together with dietary suggestions covering the treatment of certain disturbed metabolic states.

WILLARD J. STONE.

Pasadena, California
August, 1923.

INTRODUCTION

Discoveries in biological chemistry have stimulated many valuable investigations, which are not utilized as much as they should be in clinical medicine and especially in private practice. This neglect has not been peculiar to chemical methods. Many other diagnostic procedures have been less used than they should be, for similar reasons, the chief of which has been ignorance of the methods. All physicians, however, should be familiar with new methods, while those of recent laboratory training should be able to repeat them and become expert in their use. A frequent excuse for neglect has been the fear that the methods reported were imperfect and would be improved upon or modified. This has been true also of many other means of diagnosis, but should not deter the physician with the investigative turn of mind from such work, since by means of it the results can be compared, the sources of error and the fallacies recognized, and improvements adopted.

The time required for the methods, rather than the cost of apparatus or reagents, has been an important factor for the busy physician. A well-equipped small laboratory will, however, be able to do quite as satisfactory work as the large scale performance of tests in public laboratories, but with the added advantage that the physician may modify and improve his own methods and check his own work. An important reason for the wider application of chemical methods in diagnosis depends upon the importance of the findings as a guide to the condition of the body with normal or disturbed function. Many who have had such tests applied have been discouraged because diagnoses, especially names of diseases, have not been furnished. But this ignores the fact that in practice the name of the disease is not so important as accurate knowledge of the physiology of the patient; also that the changes which may occur from day to day are of more importance than the results of a

single test. So in the case of many chemical methods the course of the various changes is essential just as a temperature curve is more useful than an isolated observation of the body heat. While some of the present methods may in the light of experience be abandoned, it is certain that many others will become as necessary as the simpler clinical examinations.

Dr. Stone has given the essential details of the most valuable clinical methods of biochemistry, methods that have been extensively used by himself and others. Those already familiar with such work will find the book useful for reference, while those who have been discouraged by the mass of detail given in more exhaustive textbooks will find it a clear and accurate guide. The use of such methods of clinical study will add not only interest but greater accuracy to the work of the physician and enable him with satisfaction to take part in the general advance of clinical knowledge. The large field of clinical chemistry is open to those who are interested in giving to their patients advice founded upon facts rather than fads or fancies. It should also be recalled that many discoveries in the field of medicine have come from small laboratories, a reason which should give further stimulation to the more general adoption of such investigative methods of work.

GEORGE DOCK.

Pasadena, California
August, 1923.

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BLOOD CHEMISTRY COLORIMETRIC METHODS

CHAPTER I

Suggestions for Blood Chemistry Work

1. The colorimeter is an instrument devised for the quantitative determination of substances in solution which have developed color as a result of chemical reactions. The most important substances which may be determined by means of its use in the blood are non-protein nitrogen, urea nitrogen, uric acid, creatinin, sugar, chlorides and cholesterol.

2. For blood chemistry work the fasting state is desirable, since practically all of the established normal values have been obtained from blood specimens taken twelve to fourteen hours after the ingestion of food. If the blood specimen is taken during the period of greatest absorption from the gastro-intestinal tract, within a period of three or four hours after a meal, the figures for non-protein nitrogen, urea and sugar may be considerably increased, for which due allowance should be made in interpreting the results. As to the method of taking the blood specimen the following plan has been found satisfactory: About 6 c.c. of blood are drawn from one of the veins at the bend of the elbow by means of a dry glass syringe. One and one-half c.c. are expelled into a small tube for the routine Wassermann test while the balance is expelled into another tube containing 2 drops of a 10 per cent potassium or lithium oxalate solution to prevent clotting. This tube is inverted several times to mix thoroughly the oxalate with the blood. Denis and Beven have shown that the addition of sodium fluoride, in the proportion of 60 mg. to 10 c.c. of oxalated blood, acts as a preservative for non-protein nitrogen, urea, uric acid, creatinin and sugar for forty-eight hours at ordinary room temperature. All these constituents, except non-protein nitrogen, are preserved for periods as long as ninety-six hours.

3. To be reliable it is essential that tests always be performed under the same conditions as to fasting state and technique.

4. It has been found most satisfactory to make the tests for non-protein nitrogen, urea, creatinin, uric acid and sugar upon the blood filtrate as described in the methods of Folin and Wu. If plasma obtained from oxalated blood is used instead of whole oxalated blood, the normal value for non-protein nitrogen may be considered to be about three-quarters

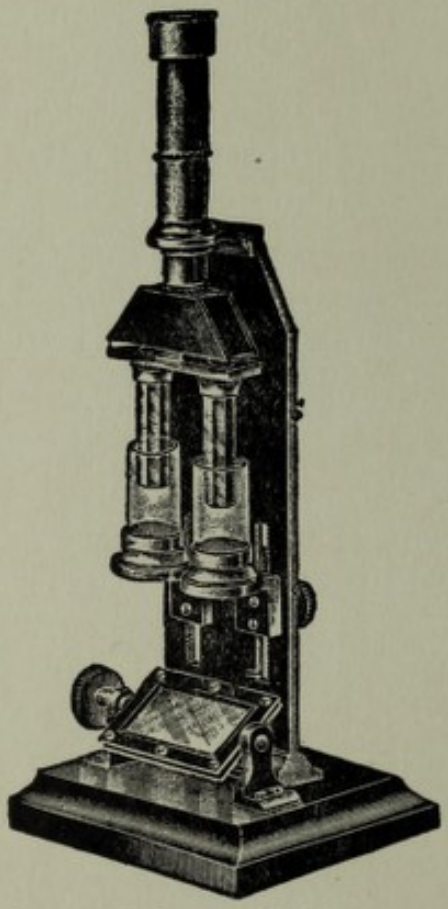

FIG. 1. Duboscq colorimeter.

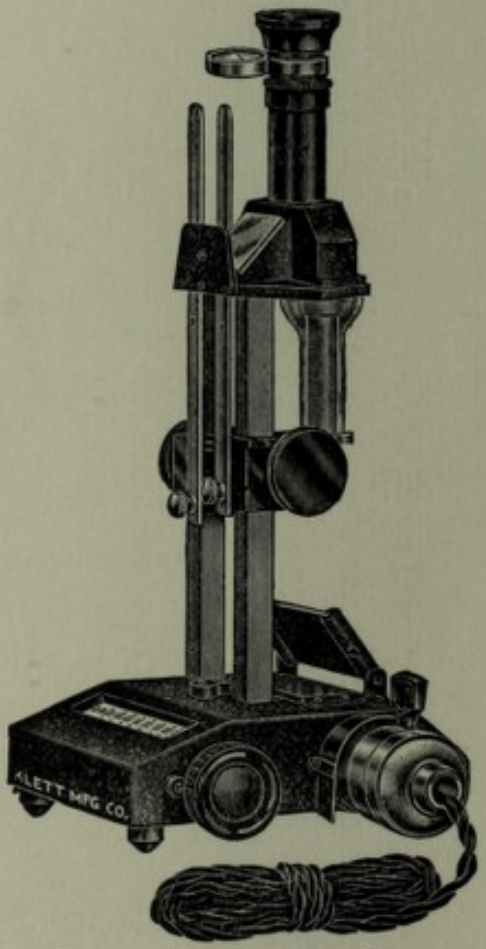

FIG. 2. Klett colorimeter.

of the value for the whole blood. The urea nitrogen and the uric acid content of plasma and of whole blood are for practical purposes almost identical. The blood serum, obtained from blood after clotting, and blood plasma contain practically identical amounts respectively of urea nitrogen and uric acid.

5. The picric acid method of Lewis and Benedict (or its modification by Myers and Bailey) for blood sugar may

give higher readings in many specimens of blood, primarily because of the absorption of creatinin by the picric acid. While not criticizing the usefulness of this test, it is believed that more uniform results are obtained by following a procedure such as that offered by the method of Folin and Wu. For verification

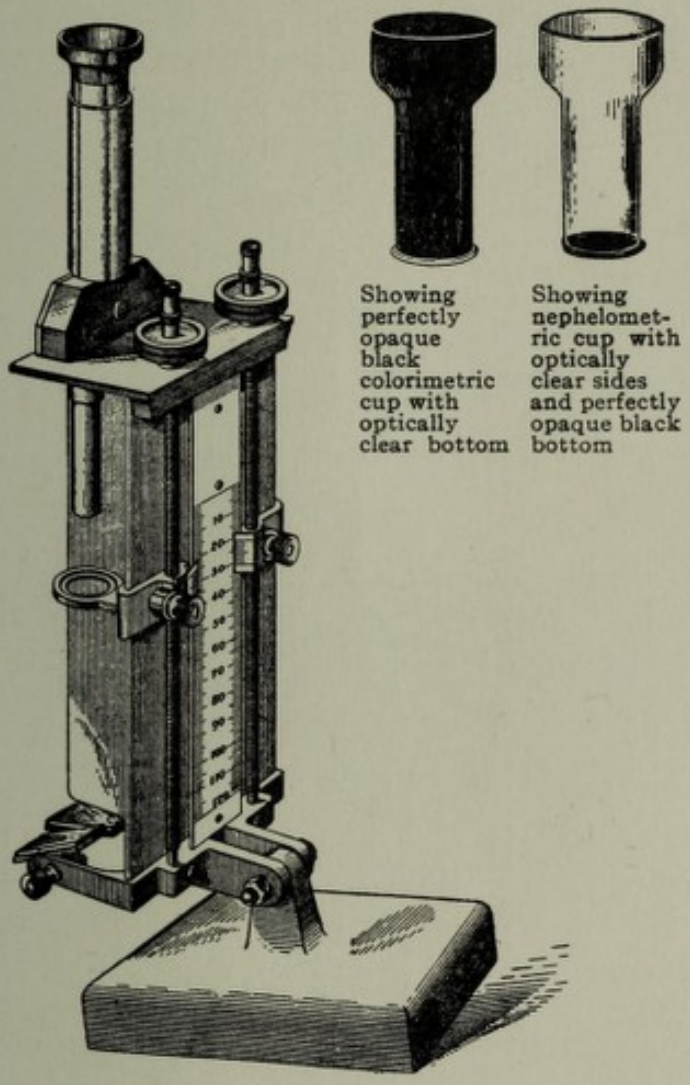

FIG. 3. Kober colorimeter.

of blood sugar in any given case the Lewis and Benedict picric-acid method is recommended.

6. Chemically pure sodium tungstate and picric acid are essential. (See notes in the text.) Some preparations of sodium tungstate are not satisfactory because of an excess of carbonate. Sodium tungstate, Primrose Brand, or Merck's c.p. sodium

tungstate are satisfactory. The Nessler's solution should be carefully prepared.

7. For standard sugar solutions, chemically pure dextroglucose (Pfanstiehl) may be recommended. The standard nitrogen, uric acid and creatinin solutions can be secured from any dealer in scientific supplies by those who do not have facilities for making their own solutions.

8. Discrepancies in the calibration of glassware, especially pipettes, burettes, graduates and volumetric flasks, should be noted when equipping the laboratory. It is best to have on

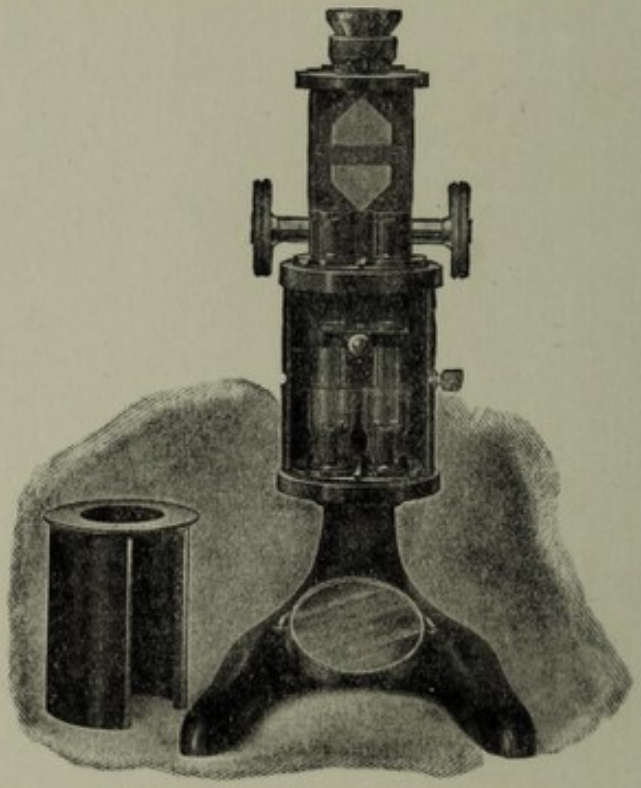

FIG. 4. Buerker colorimeter.

hand for comparison a few utensils the accuracy of which has been verified by the Bureau of Standards, Washington.

9. It is wise to calibrate frequently the colorimeter scale in order to determine that approximately identical readings are secured, using for comparison the standard solutions used in the tests. If the Kober colorimeter is used, the mirrors should be carefully adjusted to reflect the same amount of light into the cups, which should be filled about two-thirds full of the solutions. If discrepancies occur, the scale should be adjusted so that the readings coincide.

10. The blood pipettes and blood-sugar tubes of Folin, with bulb and constriction, as well as Pyrex ignition test-tubes, can be obtained from any dealer in scientific supplies.

11. The choice of the colorimeter will depend upon the individual. The Duboscq is now made in this country, as well as the Kober and the Klett model.

Formula for Calculation in Colorimeter Work

The following formula serves as the basis for computations in which different quantities of unknown and varying strengths of standard solutions are used:

$$\frac{\text{Standard Reading}}{\text{Unknown Reading}} \times \frac{\text{Strength of Standard in Mg.}}{\text{Volume of Standard as Compared with Unknown}} = \text{Mg. in Amount of Blood or Urine Used.}$$

The Normal Blood Constituents of Clinical Importance

	Per 100 c.c. of Blood
Urea nitrogen	12.0 – 15.0 mg.
Uric acid	1.5 – 4.5 mg.
Preformed creatinin	1.5 – 2.0 mg.
Total creatinin (creatin plus creatinin)	4.0 – 6.0 mg.
Amino-acid nitrogen	6.0 – 8.0 mg.
Ammonia nitrogen	0.1 – 0.2 mg.
Total non-protein nitrogen (the incoagulable nitrogen)[1]	25.0 – 35.0 mg.
Sugar	80.0 – 110.0 mg.
Cholesterol	160.0 – 200.0 mg.
Chlorides (as NaCl)	600.0 – 650.0 mg.

[1] The term non-protein nitrogen includes those nitrogenous substances remaining after precipitation of the proteins.

CHAPTER II

Preparation of Protein-Free Blood Filtrate Used in Determination of Non-Protein Nitrogen, Urea Nitrogen, Uric Acid, Preformed Creatinin, Total Creatinin, Sugar and Chlorides

CHAPTER II

Preparation of Protein-Free Blood Filtrate Used in Determination of Non-Protein Nitrogen, Urea Nitrogen, Uric Acid, Preformed Creatinin, Total Creatinin, Sugar and Chlorides (Method of Folin and Wu)

I. Solutions Used in the Preparation of Protein-Free Blood Filtrate

1. Sodium tungstate, c. p. ..... 20.0 gm.
   Distilled water to ..... 200.0 c.c.
2. *⅔ Normal Sulphuric Acid*[2]
   Sulphuric acid ..... 33.0 gm. by weight
   Distilled water to ..... 1000.0 c.c.
   This solution, while approximately correct, should be checked up by titration, since in the quantities used it is intended to be equivalent to the sodium content of the tungstate.

II. Transfer exactly 4 c.c. of the oxalated blood to 100 c.c. flask. Avoid an excess of oxalate because of interference with uric acid precipitation. Add 28 c.c. of distilled water and shake to lake the blood. Add 4 c.c. of 10 per cent sodium tungstate solution and mix. Add from graduated burette, *slowly and with constant shaking, 4 c.c. of ⅔ normal sulphuric acid.* Close flask

[2] Haden has simplified the procedure by using twelfth-normal sulphuric acid which as efficiently lakes the blood, produces more rapid filtration, gives from 10 to 15 per cent more filtrate and the filtrate is more nearly neutral. Four cubic centimeters of oxalated blood are added to 32 c.c. of twelfth-normal sulphuric acid. After laking is complete 4 c.c. of 10 per cent sodium tungstate solution are added, the mixture is well shaken and then filtered. Since the twelfth-normal acid can be made from the ⅔ normal acid by adding 125 c.c. to 875 c.c. of distilled water, the quantity of acid used in performing the test remains the same as in the original method. Forty-nine grams of sulphuric acid by weight in distilled water to 1000 c.c. makes a normal solution.

with rubber stopper and shake. The shaking should produce but little foaming. Let stand for five minutes. The color should change from red to brown. If color does not change the coagulation is incomplete, usually because of too much oxalate. In such an event add 10 per cent sulphuric acid, one drop at a time, and shake after each drop until there is no foaming and the brown coloration has occurred. It is important to avoid adding any excess of sulphuric acid beyond the amount required to secure thorough protein precipitation since an excess may also precipitate the uric acid and interfere with its subsequent determination.

Pour the mixture slowly on filter paper and cover with watch glass. The filtrate should be clear.[3] If filtrate is to be kept for a day or two add a few drops of xylol or toluol.

Ten c.c. of the blood filtrate equals 1 c.c. of blood.

Non-Protein Nitrogen

I. Solutions Used in the Determination of Non-Protein Nitrogen in the Blood

1. *Acid Phosphoric-Sulphuric Digestion Mixture*

Copper sulphate (5 per cent solution).....	50.0 c.c.
Acid phosphoric (85 per cent).....	300.0 c.c.
Acid sulphuric, c.p. (ammonia-free).....	100.0 c.c.
Distilled water.....	450.0 c.c.

Keep well stoppered to prevent absorption of ammonia from the air.

2. *Stock Nessler's Solution*

KI.....	15.0 gm.
Iodine.....	11.0 gm.

in 100 c.c. flask; add

Water.....	10.0 c.c.
Metallic mercury.....	14.5 gm.

Shake flask vigorously, using rubber cork, for seven to ten minutes until dissolved iodine has nearly all disappeared. The solution becomes quite hot. When

[3] The blood filtrate, if the correct strength of acid has been used, should be only slightly acid to Congo-red paper.

the red iodine solution has begun to pale in color, cool in running water and continue shaking until red color of iodine has been replaced by greenish color of the double iodide (about ten minutes). Separate solution from surplus mercury by decanting and washing with distilled water. Dilute solution with washings to 200 c.c.

3. *Preparation of Nessler's Reagent from Stock Nessler's Solution*

Put into 500 c.c. bottle:

10 per cent solution of NaOH	350.0 c.c.
Stock Nessler's solution	75.0 c.c.
Distilled water	75.0 c.c.

(In the preparation of the 10 per cent solution of NaOH used in Nessler's reagent it has been found much more satisfactory to make the solution accurately by weight instead of by volume.)[4]

1 c.c. contains approximately:

Hg metallic	0.1	gm.
KI	0.01	gm.
Iodine	0.001	gm.
NaOH	0.07	gm.

4. *Standard Nitrogen Solution*

Ammonium sulphate (highest purity)	0.9432	gm.
Distilled water	1000.0	c.c.

1 c.c. = 0.2 mg. nitrogen

II. Preparation of Unknown Solution

To 2.5 c.c. of filtrate, in large Pyrex ignition test-tube, add 0.5 c.c. of the acid phosphoric-sulphuric digestion mixture. Boil gently over micro-burner until water has been nearly evaporated. Cover tube with watch glass and

[4] An equal amount of water by weight may be added to sticks of sodium hydroxide. After solution allow the insoluble sodium carbonate to settle. A 10 per cent solution, free from carbonate, may then be made by dilution of the supernatant fluid. It is important to have the Nessler's solution as free from carbonate as possible.

continue boiling gently for about two minutes. Dense fumes from the acid will rise in the tube. The solution will turn dark brown and upon heating slowly will soon turn nearly colorless. Allow tube to cool. Wash contents of tube carefully with 5 to 7 c.c. of distilled water into a 25 c.c. volumetric flask. This completes the preparation of the unknown solution with the exception of the addition of Nessler's reagent. (See below.)

III. Preparation of Standard Solution

The standard usually required is about 0.2 mg. of nitrogen per 100 c.c. Place with measuring pipette 1 c.c. of standard nitrogen solution (which contains 0.2 mg. per c.c.) in 50 c.c. volumetric flask, add 1 c.c. of the phosphoric-sulphuric acid digestion mixture to balance acid in the unknown, and then add about 15 c.c. of distilled water.

IV. Final Step

Add 8 to 10 c.c. of Nessler's reagent slowly to flask containing the unknown, and when full development of color has occurred, fill to 25 c.c. mark with distilled water. Insert clean rubber stopper in flask and mix. If solution is turbid, centrifuge small portion before comparing with standard.

Add 12 to 15 c.c. of Nessler's reagent slowly to standard solution in volumetric flask and when full development of color has occurred add distilled water to 50 c.c. mark. Insert clean rubber stopper and mix. (*The Nessler's reagent should be added as nearly simultaneously as possible to unknown and standard solutions.*)

Compare unknown in colorimeter with standard set at 20 mm.

V. Calculation

The equivalent of 0.25 c.c. of blood was used; the standard solution contained 0.2 mg. nitrogen; the volume of standard was twice the volume of the unknown solution.

The calculation will be as follows, R indicating the reading of the unknown:

$$\frac{20}{R} \times \frac{0.2}{2} \text{ mg.} = \text{mg. non-protein nitrogen in } 0.25 \text{ c.c. blood}$$

or

$$\frac{800}{R} = \text{mg. non-protein nitrogen in } 100 \text{ c.c. blood.}$$

The average of many analyses of normal blood specimens has fallen between 25 and 35 mg. of non-protein nitrogen per 100 c.c. of blood.

Determination of Blood Urea Nitrogen

(Method of Folin)

I. Solutions Used

1. *Buffer Mixture to Activate the Urease Solution*

Sodium pyrophosphate (U. S. P.)..... 140.0 gm.
Glacial phosphoric acid..... 20.0 gm.

2. *Urease Solution*

Wash about 3 gm. of permutit in a flask with 2 per cent acetic acid, decant and repeat the process twice with water; add 5 gm. of Jack bean meal to 100 c.c. of 15 per cent alcohol (16 c.c. of 95 per cent alcohol plus 84 c.c. of water). Shake for ten minutes, filter and collect the filtrate in 3 or 4 different small bottles. This solution will keep for one week at ordinary room temperature, but may be preserved for four to six weeks in an ice box. Direct sunlight exposure of the solution should be avoided.

Urease Paper

Shake 15 gm. of Jack bean meal with 5 gm. of permutit and 100 c.c. of 15 per cent alcohol for about ten minutes. Filter through plicated paper and place the clear filtrate in clean flat-bottomed dishes. Pieces of ammonia-free filter paper are drawn through the

filtrate and hung up to dry, from which strips about one-half inch square may be cut for use instead of the urease solution.

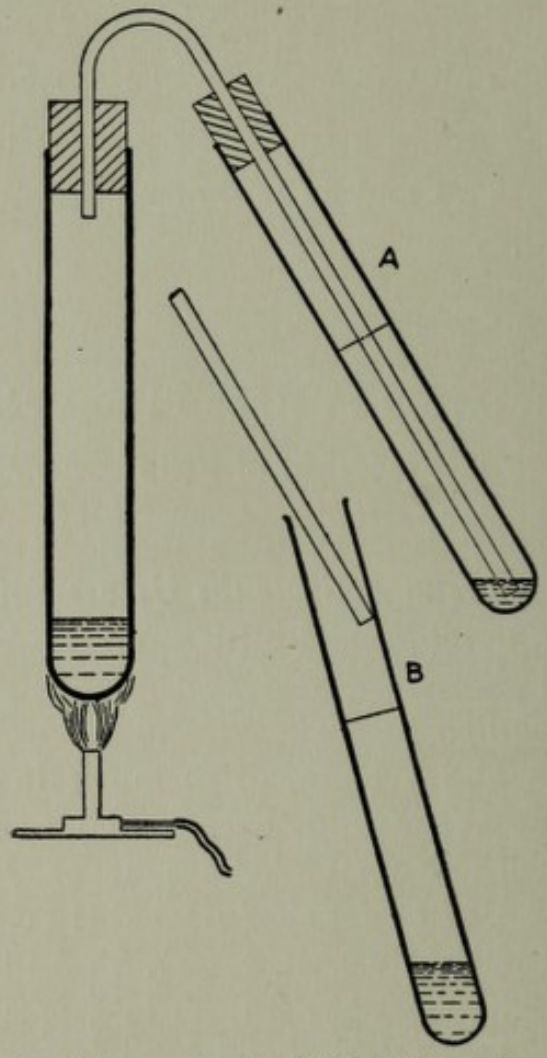

FIG. 5. A at beginning, B toward end of distillation. From Folin's "Manual of Biological Chemistry." (*Courtesy of D. Appleton and Company.*)

3. $\frac{N}{20}$ *Hydrochloric Acid*

Concentrated HCl	1.0 c.c.
Distilled water to	200.0 c.c.

(This solution is approximately correct.)

4. *Saturated Borax Solution*
5. *Paraffin Oil*

6. *Standard Nitrogen Solution*

Ammonium sulphate (highest purity).	0.9432	gm.
Distilled water.....	1000.0	c.c.

1 c.c. = 0.2 mg. nitrogen.

II. Preparation of Unknown Solution

To 2.5 c.c. of blood filtrate in a large Pyrex test-tube (which must previously have been rinsed with nitric acid and then with water if it has contained Nessler's solution), add one drop of buffer mixture and 0.5 c.c. of urease solution or a strip of urease paper. Immerse the tube in warm water (40°–55°C.) for five minutes or let stand at room temperature for fifteen minutes.

To collect the ammonia formed from the urea without using a condenser, a test-tube with perforated rubber stopper containing a curved glass tube is used for distillation as in the accompanying illustration. The distillate is collected in a graduated 25 c.c. perforated rubber-stoppered receiving tube containing 1 c.c. of $\frac{N}{20}$ hydrochloric acid solution.

Add to the blood filtrate a dry pebble, a drop or two of paraffin oil and 1 c.c. of the borax solution.[5] Boil, using a small flame at a uniform rate, for about four minutes. The boiling should not be so brisk that the emission of steam occurs from the receiving tube before three minutes. At the end of four minutes disconnect the receiving tube from the rubber stopper, let it rest in a slanting position and continue the distillation for a minute longer. Rinse out the delivery tube with a little water and after cooling the distillate with running water add the washings to it and bring the volume to 10 c.c. by adding sufficient distilled water.

III. Preparation of Standard Solution

Transfer 1 c.c. of standard nitrogen solution to a 50 c.c. volumetric flask, dilute to about 40 c.c., add about 7.5 c.c.

[5] Some of the paraffin oil used to prevent foaming is, despite usual care, carried over by steaming into the acid solution and may foul the colorimeter.

of Nessler's solution, rotate flask until nesslerization is complete and add water to the 50 c.c. mark.

IV. Final Step

Add 1.5 c.c. of Nessler's solution to the unknown and after nesslerization is complete dilute to the 12.5 c.c. mark with water.

V. Calculation

The equivalent of 0.25 c.c. blood was used; the standard solution contained 0.2 mg. nitrogen; the volume of standard was 50 c.c., while the volume of unknown was 12.5 c.c.

Compare unknown in colorimeter with standard set at 20 mm.

The calculation will be as follows, R indicating the reading of the unknown:

$$\frac{20}{R} \times \frac{0.2}{4} = \text{mg. in } 0.25 \text{ c.c. blood,}$$

or

$$\frac{400}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

Determination of Blood Urea Nitrogen (By Direct Nesslerization)[6]

I. Solutions Used

1. *Buffer Mixture* (same as in usual method).
2. *Urease Paper.*
3. *Nessler's Solution.*
4. *Standard Nitrogen Solution* (1 c.c. = 0.2 mg. nitrogen).

[6] The objections raised to direct nesslerization of the blood filtrate, because of the presence of interfering substances such as peptones and amino-acids, are not believed to be of enough importance to influence the results obtained for clinical diagnosis, since it is believed these substances are constant in both normal and pathologic blood specimens.

II. Preparation of Unknown Solution

Five cubic centimeters of blood filtrate are placed in a large test-tube (which must previously have been washed with nitric acid and then with distilled water if it has contained Nessler's solution). To this are added two drops of the buffer mixture and a strip of urease paper. The tube is then immersed in warm water at 40–55°C. for five minutes. The contents of the tube are then transferred to a 25 c.c. volumetric flask and the tube thoroughly rinsed with 10 or 12 c.c. of distilled water which is added to the contents of the flask.

III. Preparation of Standard Solution

Transfer 1 c.c. of standard nitrogen solution to a 50 c.c. volumetric flask, dilute to about 40 c.c., add about 7.0 c.c. of Nessler's solution, rotate flask until nesslerization is complete and add water to the 50 c.c. mark.

IV. Final Step

Add about 3 c.c. of Nessler's solution to the unknown solution, rotate flask, and, when nesslerization is complete, add distilled water to the 25 c.c. mark.

V. Calculation

The equivalent of 0.5 c.c. of blood was used; the standard solution contained 0.2 mg. nitrogen; the volume of the standard was 50 c.c., while the volume of the unknown was 25 c.c.

Compare unknown in colorimeter with standard set at 20 mm.

The calculation will be as follows, R indicating the reading of the unknown:

$$\frac{20}{R} \times \frac{0.2}{2} = \text{mg. in 0.5 c.c. blood,}$$

or

$$\frac{400}{R} = \text{mg. in 100 c.c. blood.}$$

Clinical Comments on Abnormal Retention of Non-Protein Nitrogen and Urea in the Blood (Azotemia)[7]

Marked increase of non-protein nitrogen (urea constituting 70–80 per cent) in the blood may be expected in partial or complete suppression of kidney function, whether the condition is acute or chronic. If complete, the condition is that usually described as uremia. It is also increased in such conditions as prostatic hypertrophy producing urinary retention and bilateral ureter compression or obstruction. The increase is especially marked in the type of nephritis brought about by poisoning with the heavy metals such as lead, arsenic and mercury. In many patients with arterial hypertension due to contracted arterioles (high diastolic blood-pressure) with its associated cardiorenal symptoms, there may be little evidence of abnormal nitrogen retention as long as the kidneys are permeable, fluids freely excreted and a low protein diet compatible with their excretory capabilities is being followed. When, however, as a result of infection, overindulgence or poisoning, the capacity of the kidneys for the elimination of waste nitrogen is overtaxed, an acute exacerbation of a preexisting nephritis, giving perhaps few symptoms, may occur, with rapid increase in the quantities of retained nitrogen in the blood.

In early kidney damage or impaired function the non-protein nitrogen of the blood is usually moderately increased, i.e., to 40–50 mg. This increase is largely made up of urea nitrogen and uric acid. During the process of digestion urea is formed in the liver from ammonia resulting from the breaking-down of the protein food constituents into amino-acids. *Urea is therefore of exogenous origin.* The uric acid results possibly from the action of enzymes or other glandular constituents upon amino and oxypurins. It is usually considered to be partly of endogenous and partly exogenous origin. In the condition described as acute nephritis the retention of non-protein nitrogen is higher, often reaching 150 mg. per 100 c.c. of blood.

[7] Azotemia, a word proposed by Widal to designate abnormal retention of nitrogen in the blood.

In the chronic types of diffuse or interstitial nephritis the retention may be less marked, especially if the patient is living within the functional capacity of his kidneys. In such chronic types the non-protein nitrogen retention will usually be found to vary from 50 to 100 mg. per 100 c.c. of blood. The types of chronic nephritis associated with severe anemia are particularly subject to blood nitrogen retention. This type of anemia, which seems to bear no constant relation to edema, is often characterized by a high hemoglobin index, approaching 1 or 1+, while the changes in the red cells usually expected in severe anemias, such as stippling and irregular shapes and sizes, are lacking. As has been suggested by Berg, this hyperchromatic type of anemia probably results from profound changes in the activity of the blood-forming organs. It is not improbable that prolonged nitrogen retention and associated acidosis may bear some etiologic relationship to it.

In the condition described as parenchymatous nephritis or "nephrosis," nitrogen retention is, except in the terminal stages of the disease, not markedly increased. In the terminal stage abnormal nitrogen retention usually occurs. In eclampsia there is usually a moderate increase in the non-protein nitrogen of the blood. This increase results from a marked retention of uric acid rather than retention of urea. The evidence points to a certain degree of impaired kidney function, not only because of the uric acid retention, but also because the threshold of sugar elimination is increased, which results in a moderate hyperglycemia. Eclampsia should not be described as a condition due to "uremia" according to knowledge of the subject now available. Disturbed kidney function, as measured by blood-chemical examinations, is not believed to be sufficient to account for the severity of the pathologic changes present in many instances of eclampsia or less severe toxemic states occurring in pregnancy. Blood-chemistry examinations should, however, always be made in such conditions in order to exclude acute nephritis with which the toxemia may be associated. In many cases of eclampsia the blood-chemistry findings may be within normal limits. In the toxemia due to nephritis

increased blood-pressures, nitrogen retention, increased uric acid and increased cholesterol values are usually found.

In many chronic conditions, such as arteriosclerosis or malignancy, abnormal blood nitrogen retention may develop as evidence of the impaired function and associated changes involving the kidneys. Such retention, if persistent and if uninfluenced by diet, undoubtedly influences the prognosis. In some severe acute infections, such as diphtheria and pneumonia, blood nitrogen retention may occur. This seems to be especially marked if dehydration and acidosis are associated conditions. In acute intestinal obstruction the retention of non-protein nitrogen or urea may reach three or four times the normal figures, an observation first noted by Tileston and Comfort.

In tetany an increase of the total non-protein nitrogen of the blood was observed, upon the onset of the attacks, in two of our patients. Haden and Orr have removed the parathyroid glands of dogs and observed that the one constant change which occurred was an increase in the total non-protein nitrogen of the blood. Such an increase is characteristic of the blood-chemical changes observed in many so-called toxemic states.

In prostatic obstruction, blood-chemistry studies are important in that knowledge of the associated kidney changes gives indication as to the most suitable time for any contemplated operation for relief. If the non-protein nitrogen figure does not exceed 35 mg. per 100 c.c., the uric acid 5 mg. and creatinin 2 mg. per 100 c.c. of blood, the patient may be considered a reasonably good operative risk. If these figures are appreciably exceeded the evidence of kidney damage should indicate caution. Institution of measures designed to relieve gradually the hydrostatic back-pressure upon the kidneys, such as preliminary bladder drainage, are of great value in restoring impaired kidney function to these patients. The retention of non-protein nitrogen, uric acid and creatinin in the blood may rapidly diminish under such treatment, the extent of the reduction depending, of course, upon the extent of the previous damage. Increased water intake greatly assists in the elimina-

tion of blood nitrogen by increasing the output of urinary nitrogen. This increased elimination includes an increase in the per cent of total nitrogen excreted as urea as well as an increase in the excretion of ammonia.

References on Non-Protein and Urea Nitrogen

Berg, B. N. *Am. J. M. Sc.*, Phila., 1922, clxiv, 88.

Berglund, H. *J. A. M. A.*, Chicago, 1922, lxxix, 1375.

Bunker, C. W. O., and Mundell, J. J. *J. A. M. A.*, Chicago, 1924, lxxxiii, 836.

Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H. *J. Exper. M.*, N. Y., 1916, xxiii, 717.

Denis, W., and Beven, J. L. *J. Lab. & Clin. M.*, St. Louis, 1924, ix, 675.

Feinblatt, H. M. *J. Lab. & Clin. M.*, St. Louis, 1923, ix, 66.

Folin, O. *Physiol. Rev.*, 1922, ii, 460.

Folin, O., and Berglund, H. *J. Biol. Chem.*, N. Y., 1922, li, 415.

Folin, O., and Denis, W. *J. Biol. Chem.*, N. Y., 1912, xi, 527; *ibid.*, 1913, xiv, 33; *ibid.*, 1916, xxvi, 491.

Folin, O., Denis, W., and Seymour, M. *Arch. Int. Med.*, Chicago, 1914, xiii, 224.

Folin, O., and Wu, H. *J. Biol. Chem.*, N. Y., 1919, xxxviii, 81.

Greene, C. H., Sandiford, K., and Ross, H., *J. Biol. Chem.*, N. Y., 1924, lviii, 845.

Haden, R. L. *J. Biol. Chem.*, N. Y., 1923, lvi, 469.

Haden, R. L., and Orr, T. G. *Am. J. M. Sc.*, Phila., 1924, clxvii, 108, 167.

Karr, W. G. *J. Lab. & Clin. M.*, St. Louis, 1924, ix, 329.

Olmsted, W. H., and Caulk, J. R. *J. A. M. A.*, Chicago, 1922, lxxix, 1380.

Plass, E. D. *J. A. M. A.*, Chicago, 1924, lxxxii, 266.

Squier, J. B., Bandler, C. G., and Myers, V. C. *J. A. M. A.*, Chicago, 1922, lxxix, 1384.

Tileston, W., and Comfort, C. W. *Arch. Int. Med.*, Chicago, 1914, xiv, 620.

Werner, E. A. The Chemistry of Urea. London, 1923.

de Wesselow, O. L. V. The Chemistry of the Blood in Clinical Medicine. Lond., 1924.

Widal, F. *Presse méd.*, Paris, 1912, xx, 973.

CHAPTER III

Uric Acid

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Uric Acid

(Method of Folin)

I. Solutions Used in the Determination of Blood Uric Acid

1. *Silver Lactate Solution* (*10 per cent*)

   Dissolve 50 gm. of silver lactate in 350 c.c. of warm water and add a mixture consisting of 50 c.c. of 85 per cent lactic acid and 50 c.c. of 10 per cent sodium hydroxide. Add water to 500 c.c. The sediment present should be allowed to settle and only the clear supernatant solution used in the test.

2. *Acidified Sodium Chloride Solution*

Concentrated HCl	1.0 c.c.
10 per cent sodium chloride sol	100.0 c.c.

3. *Lithium Sulphate Solution* (*20 per cent*)

   Dissolve 20 gm. of powdered lithium sulphate (Baker & Adamson's) in 80 c.c. of cold water. Dilute to volume of 100 c.c. and filter.

4. *15 Per Cent* (*approx.*) *Solution of Sodium Cyanide*

   Prepare enough to last three months, since this solution is believed to improve with age. Use white solid sodium cyanide not discolored or decomposed by exposure to air. Weigh out from 100 to 200 gm. of cyanide, transfer to beaker, add 6.7 c.c. of 0.1 normal sodium hydroxide solution for each gram of cyanide taken and stir until all has dissolved. The solution is opalescent. Transfer to bottle and keep at least two weeks before using. The 0.1 normal sodium hydroxide

was added to prevent decomposition with discoloration. Ammoniacal decomposition of the cyanide sooner or later destroys its efficiency, since maximum color development is retarded and turbidity is produced. This can be prevented by covering the stock bottle with a beaker instead of using a cork stopper, or by boiling off the ammonia and then diluting to the original volume. *Because of its toxicity it should, in performing the tests, be measured from a burette.*

To test for the blank due to impurity of the cyanide, transfer 5 c.c. of water, 2 drops of lithium sulphate solution and 2 c.c. of the 15 per cent cyanide solution to a test-tube. Add 1 c.c. of the uric acid reagent (given below) and let stand two minutes. The solution should remain colorless. Heat in boiling water for one and a half minutes. Some color will develop. To determine whether this color will materially affect the uric acid values in a test, repeat the above with two graduated test-tubes and with standard uric acid solution, 5 c.c. in one and 3 c.c. plus 2 c.c. of water in the other. Dilute to volume after heating and compare the colors. If the cyanide is suitable the weaker solution will give the theoretical reading, 33.5 mm., when the stronger solution is placed in colorimeter at 20 mm.

5. *Stock Uric Acid-Formaldehyde Solution*

Transfer exactly 100 mg. uric acid[8] to a funnel on a 100 c.c. volumetric flask. Dissolve 45 to 50 mg. of lithium carbonate in 15 c.c. of water by heating to about 60°C. until all the carbonate has been dissolved. With the hot carbonate solution rinse the uric acid on the funnel into its flask and shake. The uric acid will promptly dissolve. Cool flask under running water by shaking and add 40 to 50 c.c. of water. Then add 2.5 c.c. of 40 per cent formaldehyde and, after shaking to insure thorough mixing, acidify by the addition of 0.3 c.c. of glacial acetic acid. Shake to remove most of the

[8] Uric acid (Kahlbaum) is a satisfactory preparation.

carbonic acid, dilute to 100 c.c. and mix. This stock solution should be kept tightly corked in a dark bottle.

1 c.c. = 1 mg. uric acid.

6. *Standard Uric Acid Solution*

Place 1 c.c. of the stock solution, with a graduated pipette, in a 250 c.c. volumetric flask, add 125 c.c. of water and 10 c.c. of the ⅔ normal sulphuric acid used in blood protein precipitation, then add 1 c.c. of 40 per cent formaldehyde, dilute to 250 c.c. and shake to mix.

1 c.c. = 0.004 mg. uric acid.

7. *Uric Acid Reagent of Folin and Denis*[9]

In flask place

Water	75.0 c.c.
Sodium tungstate	10.0 gm.
Phosphoric acid (85 per cent)	8.0 c.c.

Partly close flask with funnel and small watch glass and boil gently for two hours, then dilute with water to 100 c.c.

II. The Test (Short Method)[10]

Have ready for use a wide-mouthed beaker containing boiling water.

Place 5 c.c. of the blood filtrate and 2 c.c. of water in a test-tube graduated at 25 c.c.

Place in a similar tube 5 c.c. of the standard uric acid solution and 2 c.c. of water.

Add 2 or 3 drops of 20 per cent lithium sulphate solution to each. The lithium sulphate solution is added to prevent precipi-

[9] Various brands of sodium tungstate may contain molybdenum in variable amounts which produces color in the presence of reducing substances. Folin and Trimble have devised a means of removing the molybdenum. For ordinary clinical work upon urine or human blood this is unnecessary since the error will not exceed 1 mg. per 100 c.c. when large traces of molybdenum are present in the tungstate.

[10] The quantity of lithium sulphate necessary to prevent precipitation, together with the precipitation which may result after boiling for eighty seconds, has been found to be such a source of trouble in some specimens of blood that the more complete "silver lactate method" given on page 30 is preferred.

tate formation in the presence of potassium oxalate used for anticlotting purposes. It may be necessary at times to add 4 drops of the lithium sulphate solution to prevent precipitation.

From the burette add 2 c.c. of 15 per cent sodium cyanide solution to each tube.

With a graduated pipette add 1 c.c. of the uric acid reagent to each tube, mix and let stand two minutes.

At the end of two minutes transfer both tubes to the boiling water for eighty seconds. Longer heating may cause precipitation.

Cool the tubes, add water to the 25 c.c. mark and mix by inverting the tubes.

III. Calculation

Read the standard against itself set at 20 mm. If the two scales do not coincide, adjust to secure correct reading.

Equal volumes of unknown and standard were used.

The equivalent of 0.5 c.c. of blood was used.

The standard contained 0.02 mg. uric acid.

With R indicating the reading of unknown the calculation will be:

$$\frac{20}{R} \times 0.02 \text{ mg.} = \text{mg. uric acid in } 0.5 \text{ c.c. blood,}$$

or

$$\frac{80}{R} = \text{mg. uric acid in } 100 \text{ c.c. blood.}$$

This short method may give values from 0.5 to 1.0 mg. higher than those obtained by the following method which is preferred.

IV. The Test (Silver Lactate Method)

Have ready for use a beaker containing boiling water.

Place 5 c.c. of the blood filtrate in a centrifuge tube, add 7 c.c. of the 10 per cent silver lactate solution. Mix and centrifuge. All the uric acid is contained in the precipitate. Decant the supernatant fluid as completely as possible.

Add 1 c.c. of the acidified sodium chloride solution to the precipitate, stir thoroughly with a glass rod, add 4 c.c. of water and, after stirring, centrifuge.

Pour the supernatant solution into a test-tube graduated at 25 c.c.

Place 5 c.c. of standard uric acid solution (containing 0.004 mg. per c.c.) in a test-tube graduated at 25 c.c.

To the contents of the unknown and to the standard tube, add 0.2 c.c. of 20 per cent solution of lithium sulphate, 2 c.c. of the 15 per cent sodium cyanide solution from burette, and 1 c.c. of the uric acid reagent. Shake each tube and let stand for two minutes.

Heat the two tubes in boiling water for eighty seconds, cool, add water in each tube to the 25 c.c. mark and compare in colorimeter as in the short method.

V. Calculation

Read the standard against itself set at 20 mm. If the two scales do not coincide, adjust to secure correct reading.

Equal volumes of unknown and standard were used.

The equivalent of 0.5 c.c. of blood was used.

The standard contained 0.02 mg. uric acid.

With R indicating the reading of unknown the calculation will be:

$$\frac{20}{R} \times 0.02 \text{ mg.} = \text{mg. uric acid in } 0.5 \text{ c.c. blood,}$$

or

$$\frac{80}{R} = \text{mg. uric acid in } 100 \text{ c.c. blood.}$$

Uric acid in the blood normally varies from 1.5 to 4.5 mg. per 100 c.c. The latter figure may be considered a high normal.

Uric Acid

(Benedict's Modification, using the blood filtrate method of Folin and Wu)

I. Solutions Used in the Determination of Blood Uric Acid

1. *Arsenic-Phosphotungstic Acid Reagent*[11]

Add 100 grams sodium tungstate, c.p., to 600 c.c. distilled water, and after dissolved add 50 gm. pure arsenic pentoxide, 25 c.c. of phosphoric acid (85 per cent) and 20 c.c. of concentrated hydrochloric acid. Boil the mixture for twenty minutes, cool and dilute to 1000 c.c. (This reagent keeps indefinitely and yields nearly seven times as much color as does the "uric acid reagent" of Folin and Denis. It is scarcely affected by polyphenols in the presence of uric acid.)

2. *5 Per Cent Sodium Cyanide Solution*

Sodium cyanide	12.5 gm.
Concentrated ammonia	0.5 c.c.
Distilled water to	250.0 c.c.

This solution should be freshly prepared once in two months. It should be measured from a burette in performing the test because of its toxicity.

3. *Stock Uric Acid Solution*

Dissolve 2.25 gm. of pure crystals of hydrogen disodium phosphate and 0.25 gm. of dihydrogen sodium phosphate in 75 c.c. of hot distilled water. Filter and make up to 125 c.c. with hot water. Pour this warm clear solution on 50 mg. pure dried uric acid (Kahlbaum) suspended in a few c.c. of water in a 250 c.c. volumetric flask. Agitate until completely dissolved and add at once exactly 0.35 c.c. of glacial acetic acid,

[11] The arsenic pentoxide used in the "arsenic-phosphotungstic acid reagent" is marketed under the name "acid arsenic c.p. powdered."

then add distilled water nearly to the 250 c.c. mark, add 1.5 c.c. of chloroform and finally add distilled water to the 250 c.c. mark. The solution should be freshly prepared every month. Before weighing it will be best to dry the uric acid at about 100°C. in an oven for an hour or two.

1 c.c. of the stock solution = 0.2 mg. uric acid.

4. *Standard Uric Acid Solution*

Measure 10 c.c. of the stock uric acid solution (containing 2 mg. uric acid) into a 500 c.c. volumetric flask and fill the flask about half full of distilled water, add 25 c.c. of dilute hydrochloric acid (one volume of concentrated acid diluted to ten volumes with distilled water) and dilute the solution to 500 c.c. This standard should be freshly prepared once in two weeks.

1 c.c. = 0.004 mg. uric acid.

II. Preparation of Unknown Solution

Transfer 5 c.c. of the blood filtrate to a graduated test-tube and add 5 c.c. of distilled water. Add 4 c.c. of the 5 per cent sodium cyanide solution.

III. Preparation of Standard Solution

Transfer 5 c.c. of the standard solution to a graduated test-tube and add 5 c.c. of distilled water. Add 4 c.c. of the 5 per cent sodium cyanide solution.

IV. Final Step

Add 1 c.c. of the arsenic-phosphotungstic acid reagent to the tube containing the unknown and to the tube containing the standard solution. Mix by inverting tubes and place them immediately in boiling water for three minutes. Then remove tubes and place in beaker of cool water for three minutes. Compare in colorimeter within five minutes because of tendency to turbidity.

V. Calculation

Equal volumes of unknown and of standard solutions were used.

The standard contained 0.02 mg. An equivalent of 0.5 c.c. of blood was used.

Therefore if S equals the height of standard solution in mm. and R equals the reading of the unknown, $\frac{S}{R} \times 0.02 \times 200$ or $\frac{S \times 4}{R}$ = mg. of uric acid per 100 c.c. of blood.

Note on the Methods for the Determination of Blood Uric Acid. From our experience and the experience of others, the values obtained on the same blood samples by the short or direct method of Folin, by the silver lactate method of Folin and by the method of Benedict show considerable variation.

The short method in our hands shows, in an average of many tests, about 1 mg. per 100 c.c. higher readings than the silver lactate method. The average normal by the short method of Folin has been found to be very close to 3.5 mg. per 100 c.c., by the silver lactate method 2.5 mg. per 100 c.c. The short method of Folin and Benedict's method have been found to give practically identical results. A comparison of the readings by the silver lactate method of Folin and the method of Benedict has shown that the silver lactate method gives slightly lower values.

Clinical Comments on Uric Acid Retention

Uric acid is probably the first non-protein nitrogen constituent to be retained in abnormal amounts by the blood in early, temporary or permanent damage to the kidneys. A distinctly higher than normal value, above 5 mg. per 100 c.c., if persistent and not remedied by appropriate dietary restrictions and other possible contributing causes, should attract attention to the possibility of kidney impairment. Such retention involves in many instances a consideration of kidney permeability and disturbed metabolism only. It will be much safer for the clinician if he does not regard uric-acid retention alone as a significant fact in the diagnosis of nephritis unless associated with abnormal retention of blood urea and creatinin. Uric acid is almost invariably high in gout, while the total non-protein nitrogen value may be within normal limits.

In acute gout the blood uric acid not uncommonly reaches 8 to 10 mg. if the patient is not on a low purine diet. In chronic gout, with which condition there is so many times evidence of associated or concomitant kidney impairment, the amount of uric acid commonly retained by the blood reaches 6 to 8 mg. per 100 c.c. This can be decreased in some instances by a low protein-low purine diet, although for many patients with chronic gout the influence of a low purine diet on the blood uric acid is not marked. The administration of uric acid eliminants, such as cinchophen and its derivatives or the salicylates, is many times of service.

In suspected gouty arthritis without tophi, but in which an increased blood uric acid figure is obtained, if the non-protein nitrogen and creatinin are also increased, gout may be excluded and the increase in uric acid be more reasonably ascribed to impaired kidney function. In non-gouty arthritis without kidney impairment the blood uric acid is usually within normal limits. Since uric acid has its origin in the body partly from endogenous and partly from exogenous sources, disturbances of metabolism resulting in retention not uncommonly lead, in those whose elimination is impaired, to muscle pains, stiffness

and headaches. If confirmation of the suspected disturbance is obtained by finding an abnormal retention of uric acid in the blood, the condition, which is usually temporary, may in many instances be relieved by a low purine diet and the administration of cinchophen or its derivatives.

Destruction of uric acid in the body takes place in all probability in the circulating blood. Temporary mobilization of an excess of uric acid may occur in the kidneys and some of the excess may be destroyed there. An excess may produce tubular damage before the level in the blood has been sufficiently decreased to remove the amounts stored in the kidneys. The recent work of Folin, Berglund and Derick has shown that the muscles and other organs, such as the liver, are practically impermeable to the soluble urates (uric acid). Uric acid destruction in the blood is slower in herbivorous than in carnivorous animals. The process by which destruction of uric acid is made possible in the blood is probably dependent upon an unknown oxidizing substance, which is supplied by the tissues or possibly by the liver. It is not believed by Folin and his associates to be an enzyme. Excretion of uric acid in man seems to depend, under physiologic conditions, upon the rate of destruction in the blood. If, however, the kidney function is disturbed, its absorbing power or ability to mobilize uric acid is decreased and its normal ratio of excretion impaired. Folin and his associates have shown that it usually takes from two to four days for the blood uric acid to reach its normal level after an injection of 20 mg. per kilo of body weight. The blood content after such an injection was increased from 5 to 15 mg. per 100 c.c.

Considerable variation occurs in the level of blood uric acid in different individuals, depending upon the power of their kidneys to absorb and eliminate uric acid. It has been shown that high protein diets increase the elimination of uric acid through the urine, which results in a lower level of blood uric acid. *In consequence more endogenous uric acid is excreted on a high protein than on a low protein diet.* In gouty individuals the kidneys apparently do not readily absorb uric acid. There

then results an abnormally high circulating uric-acid content in consequence of which more extensive destruction occurs in the blood with resulting diminished urinary excretion. The process by which uric acid is destroyed in the blood of the gouty probably differs in no essential degree from the process in normal individuals. The kidney "sensitiveness" to uric acid is, however, impaired in gout. From Folin's work it may be concluded that while the muscles of gouty individuals do not contain more uric acid than normal subjects there may be reason to believe that more dense structures, such as cartilage and connective tissue, may, because of their poor circulation, hold and mobilize quantities of uric acid which in other tissues would permit of only temporary deposition. Harding, Allin and Van Wyck have shown that 15 gm. of NaCl added to a protein or carbohydrate diet lower the level of blood uric acid. It is believed that this effect results from increased blood hydration which produces increased elimination of uric acid.

Increase of blood uric acid may occur in cancer, eclampsia, leucemia, primary anemia, polycythemia, eczema, diabetes, carbon monoxide poisoning, poisoning with the heavy metals, and in pneumonia. A marked increase occurs in starvation.

In certain skin affections, such as eczema and pruritus, both local and general, Schamberg and Brown have found the blood uric acid increased. This was especially true in eczema since 50 per cent of their patients had increased blood uric acid above the maximum normal limits, which they considered as 3.5 mg. per 100 c.c. They found a rising uric acid curve from the fortieth to the seventieth year in patients subject to chronic eczema. The average blood uric-acid figures were 25 per cent higher for the male patients than for the female patients. The blood of young patients with eczema did not as a rule contain large amounts of uric acid although there were some notable exceptions. Patients with psoriasis and urticaria had a lower average uric-acid blood content than the patients with eczema. These findings should lead one to suspect the possible relationship of increased amounts of uric acid in the blood to eczema and pruritus as a manifestation of disturbed metabolism. An appropriate low purine diet may assist the possibility of recovery.

References on Uric Acid

Baumann, Hansmann, Davis and Stevens. *Arch. Int. Med.*, Chicago, 1919, xxiv, 70.

Benedict, S. R. *J. Biol. Chem.*, N. Y., 1922, li, 187; *ibid.*, 1922, liv, 233.

Brown, H., and Raiziss, G. W. *J. Lab. & Clin. M.*, St. Louis, 1922, viii, 129.

Denis, W. *J. Biol. Chem.*, N. Y., 1915, xxiii, 147.

Feinblatt, H. M. *Arch. Int. Med.*, Chicago, 1923, xxxi, 758.

Fishberg, A. M. *Arch. Int. Med.*, Chicago, 1924, xxxiv, 503.

Folin, O., *J. Biol. Chem.*, N. Y., 1922, liv, 153.

Folin, O., and Denis, W. *J. Biol. Chem.*, N. Y., 1913, xiii, 469; *ibid.*, 1913, xiv, 32; *ibid.*, 1914, xvii, 487; *Arch. Int. Med.*, Chicago, 1915, xvi, 33.

Folin, O., Berglund, H., and Derick, C. *J. Biol. Chem.*, N. Y., 1924, lx, 361.

Folin, O., and Trimble, H. *J. Biol. Chem.*, N. Y., 1924, lx, 473.

Folin, O., and Wu, H. *J. Biol. Chem.*, N. Y., 1919, xxxviii, 81.

Gettler, A. O., and Lindemann, E. *Arch. Int. Med.*, Chicago, 1920, xxvi, 453.

Harding, V. J., Allin, K. D., and Van Wyck, H. B. *J. Biol. Chem.*, N. Y., 1924, lxii, 61.

Isaacs, R. *Arch. Int. Med.*, Chicago, 1923, xxxi, 289.

Izar, G. *Ztscbr. f. Pbysiol. Chem.*, 1911, lxxiii, 319.

Killian, J. A., and Kast, L. *Arch. Int. Med.*, Chicago, 1921, xxviii, 813.

Koehler, A. E. *J. Biol. Chem.*, N. Y., 1924, lx, 721.

Lennox, W. G. *J. A. M. A.*, Chicago, 1924, lxxxii, 602.

Lennox, W. G., and O'Connor, M. F. *J. Lab. & Clin.*, M., St. Louis, 1924, x, 99.

Lennox, W. G., O'Connor, M. F., and Wright, L. H. *Arch. Neur. & Psycbiat*, Chicago, 1924, xi, 54.

Magath, T. B. *J. Lab. & Clin. M.*, St. Louis, 1921, vi, 463.

McClure, C. W., and Pratt, J. H. *Arch. Int. Med.*, Chicago, 1917, xx, 481.

Myers, Fine, and Lough. *Arch. Int. Med.*, Chicago, 1906, xvii, 570.

Pratt, J. H. *Am. J. M. Sc.*, Phila., 1916, cli, 92. *Trans. Ass. Am. Physicians*, 1913, xxviii, 387.

Rose, M. C. *Pbysiol. Rev.*, Balt., 1923, iii, 544.

Schamberg, J. F., and Brown, H. *Arch. Int. Med.*, Chicago, 1923, xxxii, 203.

Upham, R., and Higley, H. A. *Arch. Int. Med.*, Chicago, 1919, xxiv, 557.

Williams, J. L. *Arch. Int. Med.*, Chicago, 1921, xxvii, 748.

CHAPTER IV

Preformed and Total Creatinin

CHAPTER IV

Preformed Creatinin

(Method of Folin and Wu)

I. Solutions Used in the Determination of Preformed Creatinin in the Blood

1. *Alkaline-Picrate Solution*

Saturated (1.2 per cent) picric acid[12] solution	8.5	c.c.
10 per cent sodium hydroxide solution	1.5	c.c.

2. *Stock Creatinin Solution*

Creatinin-zinc chloride	1.61	gm.
Tenth-normal HCl solution	1000.0	c.c.

1 c.c. = 1 mg. creatinin.

3. *Standard Creatinin Solution*

Stock creatinin solution	5.0	c.c.
Tenth-normal HCl solution	10.0	c.c.
Distilled water to	100.0	c.c.

Add two or three drops of xylol as a preservative.

1 c.c. = 0.05 mg. creatinin.

II. Preparation of Unknown Solution

To 5 c.c. of blood filtrate in graduated tube add 2.5 c.c. of alkaline-picrate solution. Mix and allow to stand six to eight minutes to develop color.

[12] *Picric Acid Purity.* To test purity of picric acid as used in creatinin and blood-sugar determinations, Folin and Doisy have suggested the following procedure: Add 1 c.c. of 10 per cent NaOH solution to 20 c.c. of a saturated (1.2 per cent) solution of picric acid in water. The color of the alkaline-picrate solution so prepared must not be more than twice as deep a color as that of the saturated picric acid solution. With unusually pure picric acid, the color of the alkaline-picrate solution will not be more than one and one-half times as deep as that of the picric acid solution, i.e., with picric acid solution set at 20 mm. in the colorimeter the alkaline-picrate solution will give a reading of 13 to 14 mm.

III. Preparation of Standard Solution

Measure with pipette 0.3 c.c. of standard creatinin solution (containing 0.05 mg. per c.c.) into graduated tube and add water, using same pipette, to 10 c.c. mark. Add with accurate pipette 5 c.c. of alkaline-picrate solution. Mix and let stand six to eight minutes to develop color. (The standard solution thus prepared contains 0.015 mg. creatinin.)

IV. Calculation

Note that both fields are equal when both cups of colorimeter contain the standard set at 20 mm.

The color comparison between standard and unknown should be made within fifteen minutes from the time the alkaline-picrate was added.

With standard set at 20 mm., R indicating the reading of the unknown solution, the computation will be as follows, since the equivalent of 0.5 c.c. of blood was used:

$$\frac{20}{R} \times \frac{0.015}{2} = \text{mg. in 0.5 c.c. of blood.}$$

or

$$\frac{30}{R} = \text{mg. in 100 c.c. blood.}$$

The average of many analyses has shown the normal amount of preformed creatinin to be about 1.5 mg. per 100 c.c. of blood.

Total Creatinin (Creatin plus Creatinin)

I. Solutions Used

Same as for preformed creatinin determination.

II. Preparation of Unknown Solution

Place 2 c.c. of blood filtrate in 10 c.c. volumetric flask, add 0.5 c.c. of normal hydrochloric acid. Cover mouth of flask

with tinfoil and heat in cup of boiling water for twenty minutes. Cool. Add 2 c.c. of freshly prepared alkaline-picrate solution Allow to stand five minutes and dilute with water to 10 c.c. mark.

III. Preparation of Standard Solution

Place 0.5 c.c. of the creatinin standard solution (containing 0.05 mg. per c.c.) in a 20 c.c. volumetric flask. Add 1 c.c. of normal hydrochloric acid,[13] and 4 c.c. of freshly prepared alkaline-picrate solution. Allow to stand five minutes, then add water to 20 c.c. mark. The standard so prepared contains 0.025 mg. creatinin.

IV. Calculation

Fill both colorimeter cups half full of standard solution as prepared above, and determine whether both fields are equal with tube length set at 20 mm. If both are not alike adjust vernier scale of right-hand tube by thumb-screw (Kober instrument) so that tube length corresponds to left-hand scale.

Adjust mirrors so that reflected light is equal in each field. Empty the right-hand tube and wash. Also wipe solution from plunger. Fill this tube half full of unknown solution and make comparison. R indicates reading of the unknown.

$$\frac{20}{R} \times \frac{0.025}{2} = \text{mg. in 0.2 c.c. blood,}$$

or

$$\frac{125}{R} = \text{mg. in 100 c.c. blood.}$$

The normal value for total creatinin by this method is about 6 mg. per 100 c.c. of blood.

[13] *Normal HCl.* Concentrated HCl (molecular weight 36.46) is approximately ten times a normal solution, therefore 25 c.c. conc. HCl plus distilled water to 250 c.c. constitutes a normal solution; and 25 c.c. of this latter solution plus distilled water to 250 c.c. will make a tenth-normal solution sufficiently accurate for this test.

Clinical Comments on Blood Creatinin

Creatin and its anhydride, creatinin, are products of metabolism derived from the activity of the body muscles. The importance of the metabolite, creatinin, in disturbed kidney function or disease has been recognized since the almost simultaneous publications of Folin and Denis, Shaffer, Myers and Fine, and Neubauer in 1914. It was shown by them that creatinin was increased in the blood in nephritis. This increase in nephritis is accompanied by an increase in the other non-protein nitrogenous elements of the blood, especially urea. The findings do not, however, always run parallel. *It should be recalled that the origin of creatinin is endogenous while the origin of urea is largely exogenous.* In kidney disease a low protein intake may throw less work upon these organs and the retention of urea will be diminished while the elimination of creatinin will not be affected to any great extent. As Myers has expressed it, in reference to definite kidney disease: "Apparently the kidney is never able to overcome the handicap of a high creatinin accumulation." Clinical and laboratory experience has confirmed the value of the earlier observations. From a practical standpoint it is unnecessary to estimate the quantity of creatin in the blood since the estimation of creatinin gives equally as much information.

Creatinin is considered to be the most easily eliminated non-protein nitrogen constituent of the blood under conditions of normal kidney function. For this reason considerable impairment of kidney function may exist without retention of creatinin beyond normal limits. When extensive impairment of kidney function has occurred, retention of creatinin results. An increase of preformed creatinin in the blood to 4 or 5 mg. per 100 c.c. has therefore great diagnostic and prognostic importance. Its persistence at a high level indicates severe nephritis except in prostatic or bilateral ureteral obstruction or compression. With persistent retention of 5 mg. or more per 100 c.c. of blood, few patients live longer than a few months. In acute retention due to prostatic obstruction the preformed

creatinin may reach 10 mg. with recovery when the obstruction is relieved.

Behre and Benedict have recently cast doubt upon the presence of creatinin in the blood. They believe that the blood does contain creatin, the source of which is muscle tissue and which represents in the blood a waste product for elimination by the kidneys. During the process of elimination it is converted into creatinin. They believe that it is creatin rather than creatinin which is retained in the blood when the renal function is disturbed. As a matter of correct phraseology involving physiological facts it is important that their work be confirmed. Such confirmation would not particularly change the clinical fact that creatin bodies are retained above the normal limits in the blood, particularly in the chronic forms of nephritis.

References on Blood Creatin and Creatinin

Behre, J. A., and Benedict, S. R. *J. Biol. Chem.*, N. Y., 1922, lii, 11.

Benedict, S. R. *J. Biol. Chem.*, N. Y., 1914, xviii, 183.

Chace, A. J., and Myers, V. C. *J. A. M. A.*, Chicago, 1916, lxvii, 929.

Denis, W. *J. Biol. Chem.*, N. Y., 1918, xxxv, 513.

Feinblatt, H. M. *Am. J. M. Sc.*, Phila., 1923, clxvi, 249.

Folin, O. *Ztchr. f. Physiol. Chem.*, 1904, xli, 233; *Am. J. Physiol.*, Boston, 1905, xiii, 84; *J. Biol. Chem.*, N. Y., 1914, xvii, 475.

Folin, O., and Denis, W. *J. Biol. Chem.*, N. Y., 1914, xvii, 487.

Folin, O., and Doisy. *J. Biol. Chem.*, N. Y., 1916, xxviii, 349.

Folin, O., and Wu, H. *J. Biol. Chem.*, N. Y., 1919, xxxviii, 98.

Myers, V. C., and Fine, M. S. *J. Biol. Chem.*, N. Y., 1913, xiv, 9; *ibid.*, 1913, xv, 304; *ibid.*, 1913, xvi, 169; *ibid.*, 1914, xvii, 65; *ibid.*, 1915, xx, 391; *ibid.*, 1915, xxi, 377, 383, 389, 583.

Myers, V. C., and Lough, W. G. *Arch. Int. Med.*, Chicago, 1915, xvi, 536.

Myers, V. C., and Killian, J. A. *Am. J. M. Sc.*, Phila., 1919, clvii, 674.

Neubauer, *München. med. Wchnschr.*, 1914, xi, 132.

Shaffer, P. A. *Am. J. Physiol.*, Boston, 1908, xxiii, 1; *J. Biol. Chem.*, N. Y., 1914, xviii, 525.

CHAPTER V

Blood Sugar

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Blood Sugar

(Method of Folin and Wu)

I. Solutions Used in the Determination of Blood Sugar

1. *Stock Sugar Solution, 10 mg. per c.c.*

Dextrose, c.p.	1.0 gm.
Aqueous benzoic acid solution (0.3 per cent) (as preservative) to	100.0 c.c.

2. *Standard Sugar Solution, 0.1 mg. per c.c.*

Stock sugar solution	5.0 c.c.
Aqueous benzoic acid solution (0.3 per cent) (as preservative) to	500.0 c.c.

3. *Molybdate-Phosphate Solution*

Molybdic acid (85 per cent) c.p.	17.5 gm.
Sod. tungstate, c.p.	2.5 gm.
10 per cent sod. hydrate sol.	100.0 c.c.
Distilled water	100.0 c.c.

Boil vigorously for twenty to thirty minutes to remove ammonia, cool, dilute to about 175 c.c., add 62.5 c.c. of concentrated (85 per cent) phosphoric acid, then dilute to 250 c.c.

4. *Alkaline Copper Tartrate Solution*

Anhydrous sod. carbonate, c.p.	20.0 gm.
Distilled water	200.0 c.c. in flask;
Add	
Tartaric acid	3.75 gm., and when dissolved

Add	
Crystallized copper sulphate.....	2.25 gm.
Mix and make volume to.....	500.0 c.c.

II. Preparation of Unknown Solution

Place 2 c.c. of the blood filtrate in a Folin blood-sugar tube graduated at 25 c.c. Add 2 c.c. of alkaline copper tartrate solution. The surface of the mixture should reach the constricted part of the tube. If the bulb is too large for the volume (4 c.c.), not more than 0.5 c.c. of a diluted 1 to 1 alkaline copper tartrate solution may be added. If this does not suffice to bring the contents to the narrow part, the tube should be discarded; likewise, if the bulb is so small that 4 c.c. fill it above the neck, the tube should be discarded.

III. Preparation of Standard Solution

Place in another similar tube 2 c.c. of standard sugar solution equal to 0.2 mg. of dextrose and add 2 c.c. of the alkaline copper tartrate solution.

IV. Final Step

Place the two tubes in a cup of boiling water for six minutes. Then place tubes in a cup of tap water to cool for two or three minutes.

Add to each tube 2 c.c. of the molybdate-phosphate solution which dissolves the cuprous oxide usually within two minutes. When dissolved, dilute the resulting blue solutions in the tubes to the 25 c.c. mark, insert a rubber stopper and invert the tubes to mix. This should be done carefully, since the greater part of the blue color has been formed in the bulb of the tube.

It is important that the unknown and the standard tubes be heated the same length of time, and also that they be approximately the same temperature when the molybdate-phosphate solution is added. In this method reoxidations of the cuprous compounds are excluded, the blank due to blue alkaline copper

tartrate is eliminated, and the error due to so-called phenols in the blood filtrate is removed.

V. Calculation

The standard should be set at 10 mm.

Equal volumes of unknown and standard solutions were used.

The standard contained 0.2 mg. of dextrose.

The equivalent of 0.2 c.c. of blood was used.

The calculation will therefore be as follows, R indicating the reading of the unknown:

$$\frac{10}{R} \times 0.2 \text{ mg.} = \text{mg. in } 0.2 \text{ c.c. blood,}$$

or

$$\frac{1000}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

The average of many analyses of normal blood specimens has fallen between 80 and 110 mg. of sugar per 100 c.c.

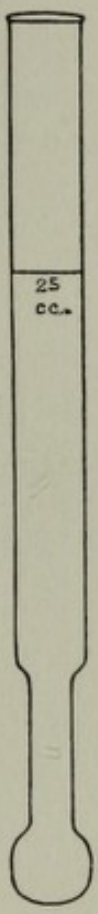

Fig. 6. Folin Blood-Sugar Tube.

Blood Sugar

(Picric Acid Method)

(Modified from the Lewis-Benedict and Myers-Bailey Methods)

I. Solutions Used in the Determination of Blood Sugar by the Picric Acid Method

1. *Hydrochloric Acid, 2.5 per cent Solution*

Acid hydrochloric, conc.	2.5 c.c.
Distilled water to	100.0 c.c.

2. *Picrate-Picric Acid Solution*

Dry powdered picric acid, c.p.	36.0 gm.
Sodium hydroxide, 1 per cent solution	500.0 c.c.
Hot water	400.0 c.c.

Shake until dissolved and when cool add distilled water to 1000 c.c.

3. *Myers-Bailey Picric Acid Sugar Standard*

Dextro-glucose, anhydrous, c.p. ..... 0.01 gm.
Picric acid, c.p., saturated sol. in water.. 100.0 c.c.
1 c.c. = 0.1 mg. sugar.
(This solution keeps indefinitely.)

4. 20 *Per Cent Sodium Carbonate Solution*

Sodium carbonate, anhydrous, c.p..... 20.0 gm.
Distilled water to..... 100.0 c.c.

II. Preparation of Unknown Solution

Place 1 c.c. of oxalated blood in 15 c.c. tube; place 2 c.c. of 2.5 per cent hydrochloric acid solution in a small graduate and rinse pipette by drawing the solution up in it two or three times to remove blood adhering to wall; add the washings to the blood and shake to lake it.[14] Then add exactly 7 c.c. of picrate-picric acid solution. Insert cork and shake to mix thoroughly. Filter through paper.

Place 3 c.c. of the filtrate (equal to 0.3 c.c. blood) in graduated tube and add 1 c.c. of 20 per cent sodium carbonate solution.

III. Preparation of Standard Solution (Myers and Bailey)

Place in graduated tube 3 c.c. of the Myers-Bailey picric acid sugar standard; add 1 c.c. of 20 per cent sodium carbonate solution. This standard contains 0.3 mg. glucose.

IV. Final Step

Place in cup of boiling water for ten minutes both the unknown and standard tubes. Cool the tubes to room tempera-

[14] Two c.c. of 2.5 per cent solution HCl, when added to 1 c.c. of blood when final mixture equals 10 c.c. will bring the acid dilution to 0.05 normal, which amount Benedict has shown is necessary, for proper precipitation of the proteins, in addition to the picrate-picric acid solution used for that purpose. Three c.c. of the 2.5 per cent HCl will be necessary if 2 c.c. of blood are used and the final dilution equals 15 c.c.

ture and then add distilled water to each to the 10 c.c. mark. Allow ten minutes for the development of color and then compare in the colorimeter with standard set at 15 mm.

V. Calculation

R indicates the reading of the unknown.

$$\frac{15}{R} \times 0.3 \text{ mg.} = \text{mg. in } 0.3 \text{ c.c. blood,}$$

or

$$\frac{1500}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

Micro Method for Blood Sugar[15,16]

I. Solutions Used in the Determination

(Same as in Folin and Wu method)

II. Preparation of Unknown Solution

Prior to drawing blood from the finger or ear draw up in pipette a few drops of a strong solution of potassium oxalate. Expel by blowing through pipette. A sufficient quantity remains adherent to the walls to prevent coagulation while securing the blood specimen.

Draw 0.1 c.c. of blood from finger by means of an accurate pipette. Expel blood into graduated centrifuge tube containing 0.8 c.c. of twelfth-normal sulphuric acid to lake the blood. Draw up some of the solution in pipette two or three times to remove completely all of blood adherent to the wall. Add 0.1 c.c. of 10 per cent sodium tungstate solution. Roll the tube between palms of hands until mixture changes to a dark brown color. Allow to stand five minutes. Add 1.0 c.c. distilled water. Insert cork

[15] In this micro method the proportion of tungstate and acid to total volume, as well as the proportion in the amount taken for analysis, is preserved.

[16] Finger or capillary blood if obtained under fasting conditions may be regarded as containing substantially the same sugar content as venous blood, but if obtained from subjects who are not fasting, as in sugar tolerance tests, such an assumption is probably not true.

and shake thoroughly, then centrifuge at moderately high speed for five minutes. Remove with a pipette 1.0 c.c. of the supernatant fluid, equal to 0.05 c.c. blood, and transfer to a minimized Folin blood sugar tube (graduated at 6.0 and 8.0 c.c.). Add 1.0 c.c. of the alkaline-copper tartrate solution.

III. Preparation of the Standard Solution

Place in a minimized Folin blood-sugar tube 1.0 c.c. of the standard sugar solution (equal to 0.1 mg. of dextrose) and add 1.0 c.c. of the alkaline-copper tartrate solution.

IV. Final Step

Transfer the tubes containing the unknown and the standard solutions to a cup of boiling water for six minutes. Then place tubes in a cup of tap water for two or three minutes to cool.

Add 1.0 c.c. of the molybdate-phosphate solution to each tube. When full development of color has occurred add distilled water in the tube containing the unknown to the 8 c.c. mark and distilled water in the standard tube to the 8 c.c. mark. Insert a rubber stopper in each tube and invert several times to thoroughly mix the colors.

V. Calculation

The unknown should be read in the colorimeter against the standard set at 10 mm.

The unknown 1.0 c.c. of fluid removed after the precipitation contains the equivalent of 0.05 c.c. of blood.

The standard contains 0.1 mg. of dextrose.

The volume of standard was equal to that of the unknown.

With R indicating the reading of the unknown the calculation will be as follows:

$$\frac{10}{R} \times 0.1 \text{ mg.} = \text{mg. sugar in } 0.05 \text{ c.c. blood,}$$

or

$$\frac{2000}{R} = \text{mg. sugar in } 100 \text{ c.c. of blood.}$$

Clinical Comments on Blood Sugar

In diabetes occurring in patients with impaired kidney function the non-protein nitrogen constituents of the blood may also be increased in addition to the increase in blood sugar. The threshold of sugar excretion in the urine may also be higher. The renal threshold for sugar excretion, however, varies within quite wide limits. It has been believed that with so-called normal kidneys sugar will appear in the urine when the blood sugar reaches 170 to 175 mg. per 100 c.c. In diabetics of long standing, sugar may be absent in the urine when the blood sugar has reached 250 to 350 mg. per 100 c.c., due to the associated nephritis. In early diabetes the blood will be found to contain from 160 to 200 mg. of sugar per 100 c.c. The threshold of sugar excretion in the urine may be unimpaired when the blood sugar is only moderately increased to 160 to 170 mg. and no sugar may be found in the urine. It is important, however, to recognize in such patients the probability that an early diabetic state exists, regardless of the absence of sugar in the urine, since so much may be accomplished for their benefit by appropriate dietary restrictions. In any doubtful case it is important to examine several urinary specimens passed one hour after hearty meals.

For individuals with lowered ability to metabolize carbohydrates it is important to determine their capacity by performing the so-called glucose tolerance test. This consists in determining the sugar content of the blood in the fasting state (before breakfast). A urinary specimen is to be taken at this time. The patient is then given 100 gm. of anhydrous glucose dissolved in 250–300 c.c. of iced water to which the juice of a lemon has been added. Specimens of blood are taken for the determination of sugar at the end of one hour, two hours, and three hours. At the end of each of these periods the patient empties the bladder. Each specimen of urine is examined for sugar, and if found to be present the percentage is estimated in the polariscope or by means of Benedict's quantitative method. The

patient should drink about 200 c.c. of water each hour while the test is in progress.

The interpretation of the findings may be summarized as follows: For the normal individual the blood sugar is increased during the first hour after taking the glucose, reaching its maximum at that interval and falling again to normal at the end of two hours. In the pre-diabetic state the rise in blood sugar is most marked at the end of the first hour, but approaches the normal level more slowly at about the end of the three hour interval or longer. In the diabetic state the rise of the blood-sugar level is more slowly reached, the maximum amount being found in the blood at the end of two or three hours. The normal level (equal to the amount of sugar in the blood prior to the test) is reached more slowly, usually at the end of five to eight hours.

One of the most important functions of the liver is that of regulating the amount of blood sugar. If the liver of the dog is removed, or the liver and pancreas together, a hypoglycemia occurs. Mann has shown that the glycogen stored in the body is divided about equally between the liver and the muscles.

In so-called "renal diabetes," a rare condition, the blood sugar may be normal while the urine contains glucose which persists and *is unaffected by carbohydrate restrictions*. The kidneys are more permeable to sugar excretion than normal, that is, the "threshold" for sugar excretion is below the level of the normal sugar in the blood. The renal diabetic is able to utilize sugar for his body needs the same as a normal individual.

In the condition known as "alimentary glycosuria" sugar may be found in the urine during the period of alimentary absorption after a meal rich in carbohydrates. The sugar will not be found in the urine during the fasting state ten to twelve hours after a meal.

In states of exhaustion brought about by acute and chronic infections it has been believed that a lowered blood sugar occurred. Such has been shown to be the case after marked physical exertion in Marathon runners by Levine. The exhaustion shown by them was not unlike the hypoglycemic shock

seen after insulin overdosage. The administration of dextrose by vein has been advocated in the treatment of such acute infections as pneumonia, on the basis that the patient's resisting powers to the infection might be increased. Dr. Norman J. Kilborne, when on duty in the pneumonia wards, made for me a series of blood-sugar estimations on 14 patients with pneumonia. Five patients had confluent bronchopneumonia while 9 had lobar pneumonia. The lowest blood sugar obtained was 90 mg., the highest 181 mg. per 100 c.c. The average for the 14 patients was 123 mg. per 100 c.c. But one death occurred in the series in a patient with coexistent lues and chronic pulmonary disease, in whom the blood sugar estimation showed 133 mg. There was no evidence, among the patients who recovered, that higher than average blood sugars had anything to do with their recovery.

On the other hand it has been shown that in heart muscle failure from toxemia with collapse symptoms and low blood pressures, such as may occur in acute sepsis, diphtheria and scarlatina, the intravenous injection of 50 to 200 c.c. (depending upon body weight) of 10 per cent glucose solution renders the heart more capable of responding to the action of stimulating drugs such as digitalis, pituitrin or epinephrin. Edmunds has been able to resuscitate dogs with marked heart muscle failure, after the injection of fatal doses of diphtheria toxin, by the intravenous injection of 10 per cent glucose solution followed by the injection of digitalis, pituitrin and epinephrin.

Thalhimer, and Fisher and Snell have shown that in preoperative and postoperative non-diabetic acidosis, as well as in shock and in the acidosis resulting from the toxemic vomiting of pregnancy and eclampsia, injections of 1000 c.c. of a 10 per cent solution of glucose given slowly in the vein have been followed by prompt relief of the symptoms when insulin was also injected subcutaneously in doses equal to 1 unit of U-20 for each 3.0 gm. of injected glucose.

References on Blood Sugar

Baumann, E. J., and Isaacson, R. L. *J. Lab. & Clin. Med.*, St. Louis, 1922, vii, 357.

Benedict, S. R. *J. Biol. Chem.*, N. Y., 1918, xxxiv, 203; *ibid.*, 1919, xxxvii, 503.

Byrd, T. L. *J. A. M. A.*, Chicago, 1924, lxxxiii, 508. *Wis. M. J.*, Milwaukee, 1925, xxiii, 492.

Edmunds, C. W. *Tr. Ass. Am. Phys.*, Wash. meeting, 1925.

Fisher, D., and Snell, M. *Wisconsin M. J.*, Milwaukee, 1923, xxii, 220; *J. A. M. A.*, Chicago, 1924, lxxxii, 699; *ibid.*, 1924, lxxxiii, 1906.

Folin, O., and Wu, H. *J. Biol. Chem.*, N. Y., 1919, xxxviii, 81; *ibid.*, 1920, xli, 367.

Gray, H. *Boston M. & S. J.*, 1923, clxxx, 168. *Arch. Int. Med.*, Chicago, 1923, xxxi, 241; 259.

Höst, H. F., and Hatlehol, R. *J. Biol. Chem.*, N. Y., 1920, xlii, 347.

Hagedorn, H. C., and Jensen, B. N. *Biochem. Zeitschr.*, 1923, cxxxv, 46.

Janney, N. W. *J. Biol. Chem.*, N. Y., 1915, xx, 321; *Arch. Int. Med.*, Chicago, 1916, xviii, 584.

Janney, N. W., and Blatherwick, N. R. *J. Biol. Chem.*, N. Y., 1915, xxiii, 77.

Janney, N. W., and Csonka, F. A. *J. Biol. Chem.*, N. Y., 1915, xxii, 203.

Janney, N. W., and Isaacson, V. I. *Arch. Int. Med.*, Chicago, 1918, xxii, 160.

John, H. J. *J. Metab. Research*, Morristown, 1922, i, No. 4.

Levine, S. A., Gordon, B., and Derick, C. L. *J. A. M. A.*, Chicago, 1924, lxxxii, 1778.

Lewis, R. C., and Benedict, S. R. *J. Biol. Chem.*, N. Y., 1915, xx, 61.

Linder, G. C., Hiller, A., and Van Slyke, D. D. *J. Clin. Investig.*, Balt., 1925, i, 247.

Mann, F. C. *Tr. Sect. Path. & Physiol., A. M. A.*, 1925.

Myers, V. C. Pract. Chem. Analysis of Blood, St. Louis, 1921.

Myers, V. C., and Bailey, C. V. *J. Biol. Chem.*, N. Y., 1916, xxiv, 147.

Pollock, H. O., and McEllroy, W. S. *Am. J. M. Sc.*, Phila., 1922, clxiii, 571.

Randles, F. S., and Grigg, W. K. *J. A. M. A.*, Chicago, 1924, lxxxii, 684.

Rowe, A. W. *Arch. Int. Med.*, Chicago, 1924, xxxiv, 388.

Rowe, A. W., and Chandler, J. *Endocrinology*, Los Angeles, 1924, viii, 803.

Sherrill, J. W., and John, H. J. *J. Metab. Research*, Morristown, 1922, i, No. 1.

Thalhimer, W. *J. A. M. A.*, Chicago, 1923, lxxxi, 383; *ibid.*, 1924, lxxxii, 696.

Wilder, R. M., Boothby, W. M., and Beeler, C. *J. Biol. Chem.*, N. Y., 1922, li, 311.

Wilder, R. M., and Winter, M. D. *J. Biol. Chem.*, N. Y., 1922, lii, 393.

CHAPTER VI

Blood Chlorides

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Blood Chlorides

(Method of Whitehorn, using the protein-free filtrate of Folin and Wu)

I. Solutions Used in the Determination of Blood Chlorides

1. *Silver Nitrate Solution*

   Dissolve 4.791 gm. of c.p. silver nitrate in distilled water and add up to the 1000 c.c. mark in volumetric flask. Preserve in dark bottles. 1 c.c. = 1 mg. Cl.

2. *Sulphocyanate Solution*

   This should be prepared volumetrically. Add about 3 gm. of potassium sulphocyanate or 2.5 gm. of ammonium sulphocyanate to 1000 c.c. of distilled water. By titration and dilution the solution should be standardized so that 5 c.c. are equivalent to 5 c.c. of the silver nitrate solution.

3. *Nitric acid*, concentrated, of a specific gravity of 1.42.

4. *Sodium tungstate*, used in the preparation of the protein-free blood filtrate. This should be free from chlorides. To test, mix one volume of sodium tungstate solution with two volumes of concentrated chloride-free nitric acid and filter into a test-tube containing silver nitrate solution. Turbidity indicates contamination with halogen.

II. Method

Accuracy is especially important in measuring the solutions, since slight variations in the amount of chlorides may be of significance. Volumetric flasks are recommended for the 1–10 dilution.

With a pipette place 10 c.c. protein-free filtrate (equivalent to 1 c.c. blood) in a porcelain dish. Add 5 c.c. of the standard

silver nitrate solution and stir thoroughly. Then add 5 c.c. of concentrated nitric acid, mix by stirring and let stand five minutes. Then add with a spatula an abundant amount (about 0.3 gm.) of powdered ferric ammonium sulphate as indicator.

Titrate the excess of silver nitrate with the standard sulphocyanate solution until the definite salmon red (not yellow) color of the ferric sulphocyanate persists when stirred for a few seconds.

III. Calculation

Each cubic centimeter of the sulphocyanate solution used in titration is equivalent to 1 c.c. of the silver nitrate solution. The difference between the number of cubic centimeters of silver nitrate solution taken and the excess by titration, i.e., 5 minus the number of cubic centimeters of sulphocyanate solution titrated, will represent the volume which reacted with chloride in the ratio of 1 c.c. to 1 mg. of Cl.

Since 1 c.c. of blood was used, the calculation will be 5 minus the number of cubic centimeters of standard sulphocyanate solution used = mg. of Cl per cubic centimeter of blood. To convert Cl figures into NaCl divide by 0.606. Multiply by 100 to obtain the result per 100 c.c. of blood.

Example: It required by titration 1 c.c. of standard sulphocyanate solution to produce the end reaction. The result would be 5 minus 1 = 4 mg. of Cl per cubic centimeter of blood, or 6.6 mg. sodium chloride per cubic centimeter or 660 mg. per 100 c.c. of blood.

Blood Chlorides

(Method of Rieger, using the protein-free filtrate of Folin and Wu and based upon the principle of Rappleye)

I. Solutions Used in the Determination of Blood Chlorides

1. A sodium tungstate solution prepared as follows to remove chlorides: Prepare a 10 per cent solution of sodium tungstate and after acidifying with an equal volume of concentrated nitric acid filter off the lemon-yellow precipitate. To the filtrate, if clear after the addition of a few more drops of nitric acid, a few drops of silver nitrate test solution are added, which should not be turbid when viewed by transmitted light if free from chlorides. To purify the tungstate solution it is poured into a cylinder containing an equal volume of 50 per cent sulphuric acid. The precipitate is allowed to settle and the supernatant fluid is then siphoned or poured off. The precipitated acid is then washed by decantation until the test for chlorides is no longer given. The precipitate is then dissolved in the requisite amount of 40 per cent sodium hydroxide, using 7 c.c. for each 10 gm. of sodium tungstate taken. The reaction of the resulting solution should be adjusted with dilute sulphuric acid until neutral to litmus. Enough water is then added to make a solution with sp. gr. of 1.15. This is filtered and is then ready for use as a 10 per cent neutral chloride-free solution of sodium tungstate.

2. *Standard Silver Solution*

Silver nitrate crystals	7.2653	gm.
Nitric acid, sp. gr. 1.42	250.0	c.c.
Sat. sol. iron ammonium alum	50.0	c.c.
Distilled water to	1000.0	c.c.

3. *Ammonium Sulphocyanate Solution*

Ammonium sulphocyanate	0.75	gm.
Distilled water	1000.0	c.c.

This should be adjusted by titration so that 25 c.c. equals 5 c.c. of the silver solution.

4. *Sulphuric Acid ⅔ Normal Solution*

Sulphuric acid..... 35.0 gm. by weight
Distilled water to..... 1000.0 c.c.
This solution is approximately correct.

II. Method

Place 5 c.c. of the sodium tungstate solution in a 50 c.c. volumetric flask, add 5 c.c. of oxalated blood and 5 c.c. of ⅔ normal sulphuric acid. The flask is well agitated and allowed to stand for one hour. Distilled water is then added to the 50 c.c. mark, the flask agitated and the contents filtered. The filtrate should be water white and give no precipitate with an equal volume of nitric acid (absence of tungstate). The presence of tungstate greatly obscures the end point in the succeeding titration.

Twenty c.c. of the filtrate, which represent 2 c.c. of blood, are placed in a 50 c.c. volumetric flask. To this are added 10 c.c. of distilled water and 10 c.c. of the standard silver solution. Distilled water is then added to the 50 c.c. mark. The flask is shaken vigorously to coagulate the silver chloride. The suspension is then filtered. Twenty-five c.c. of the filtrate are then titrated with the ammonium sulphocyanate solution to the appearance of the first brown tinge. The reaction is quite sharp.

III. Calculation

The number of cubic centimeters of sulphocyanate solution used to secure the reaction is subtracted from 25. The difference is then multiplied by 50 to obtain the number of milligrams of sodium chloride per 100 c.c. of whole blood.

Example. It required 12.2 c.c. of sulphocyanate solution by titration to secure the reaction of a brown tinge to the silver chloride filtrate. Subtracting this from 25 would equal 12.8, which, multiplied by 50, would equal 640 or the number of mg. of sodium chloride per 100 c.c. of blood.

Clinical Comments on Blood Chlorides

Under normal conditions whole blood contains about 650 mg. chlorides (as sodium chloride) per 100 c.c. The chloride content of blood cells is approximately half that of blood serum. Gram's figures give 186 mg. per 100 c.c. for the cells and 357 to 381 mg. per 100 c.c. for the serum. The chloride content of whole blood will thus largely depend upon the cell volume and will be higher in those with marked anemia. When whole blood is exposed to the air, with loss of carbon dioxide, the amount of chloride present (as sodium chloride) was found by Gram and Norgaard to average about 610 mg. per 100 c.c. In chronic nephritis the content may vary between 450 and 750 mg., depending upon the ability of the kidneys to excrete. In the acute nephritis of mercury poisoning the blood chlorides are usually below the normal average. The reason for this has not been satisfactorily explained. In acute nephritis due to other causes the blood chlorides are increased. In diabetes mellitus and insipidus the chloride content of the blood is decreased because of the diuresis. In edema associated with cardiac and renal disease the chloride concentrations are usually increased because of the absence of diuresis. This is especially true in that form of kidney disease designated as chronic parenchymatous nephritis or "nephrosis." In pneumonia the blood chlorides are relatively low and correspond with the lowered urinary chloride excretion in this disease. Peabody has shown that chlorine, sodium and calcium are retained by the body in pneumonia, while there is no retention of potassium or magnesium. Since the chlorides of the blood are low and the excretion of chlorides scant in pneumonia, mobilization or retention must occur throughout the tissues. No satisfactory explanation has been offered for this phenomenon.

Ramirez and his associates, using the method of Friend, examined the blood of asthmatics, those with eczema and those with urticaria as to the chloride content. They found an average blood chloride content among 18 asthmatics of 614 mg. per 100 c.c., among 8 patients with urticaria of 605 mg. per

100 c.c., and among 4 patients with eczema of 642 mg. per 100 c.c.

In intestinal obstruction of the upper tract, MacCallum and his associates showed in 1920 that a fall in the blood chlorides occurred together with diminished excretion of chlorides in the urine and an increase in the carbon-dioxide combining power of the blood plasma. These changes occurred coincident with the rise of non-protein and urea nitrogen in the blood, an observation first noted by Tileston and Comfort in 1914 and confirmed experimentally by Cooke, Rodenbaugh and Whipple in 1916. In intestinal obstruction of the upper tract the absorbed toxins produce marked destruction of tissue proteins which may lead to marked renal involvement. With this change a high carbon-dioxide plasma combining power, an alkalosis, is usually associated. The condition clinically is thus seen to be quite different from the acidosis resulting from dehydration and vomiting. Haden and Orr in 1923 directed attention to the value of injections of sodium chloride in upper intestinal tract obstruction. They have recommended the administration of 3 per cent sod. chloride solution, in proportion of 1 gm. per kilo of patient's weight, by hypodermoclysis or intravenous injection daily until the blood nitrogen content is decreased to normal and the blood chloride content increased.

Convulsions, due to tetany with marked alkalosis, not infrequently occur in association with obstruction at the pylorus. The use of sodium chloride solution subcutaneously as above outlined, prior and subsequent to gastroenterostomy, or the intravenous injection of Ringer's solution containing an additional gram of calcium chloride (for an adult), or similar medication through a duodenal or enterostomy tube, is indicated in such conditions.

References on Blood Chlorides

Bang, I. Microbestimmung einiger Blutbestandteile, Wiesbaden, 1916, p. 6.

Dixon, C. F. *J. A. M. A.*, Chicago, 1924, lxxxii, 1498.

Folin, O., and Wu, H. *J. Biol. Chem.*, N. Y., 1919, xxxviii, 81.

Fridericia, L. S. *J. Biol. Chem.*, N. Y., 1920, xlii, 245.

Friend, H. *J. Biol. Chem.*, N. Y., 1922, li, 115.

Gram, H. C. *J. Biol. Chem.*, N. Y., 1923, lvi, 593.

Gram, H. C., and Norgaard, A. *J. Biol. Chem.*, N. Y., 1923, lvi, 429.

Haden, R. L., and Orr, T. G. *Johns Hopkins Hosp. Bull.*, Balt., 1923, xxxiv, 26; *J. Exper. M.*, N. Y., 1923, xxxvii, 365, 377; *ibid.*, 1923, xxxviii, 55, 477; *Surg., Gynec. & Obst.*, Chicago, 1923, xxxvii, 465; *J. A. M. A.*, Chicago, 1924, lxxxii, 1515; *J. Exper. M.*, N. Y., 1924, xxxix, 321; *ibid.*, 1925, xli, 107, 113, 119.

Höst, H. F. *J. Lab. & Clin. M.*, St. Louis, 1920, v, 713.

MacCallum, W. B., and Associates. *Johns Hopkins Hosp. Bull.*, Balt., 1920, xxxi, 1.

McLean, F. C. *J. Exper. M.*, N. Y., 1915, xxii, 212.

McLean, F. C., and Van Slyke, D. D. *J. Biol. Chem.*, N. Y., 1915, xxi, 361.

Myers, V. C., and Short, J. J. *J. Biol. Chem.*, N. Y., 1920, xliv, 47.

Norgaard, A., and Gram, H. C. *J. Biol. Chem.*, N. Y., 1921, xlix, 263.

Peabody, F. H. *J. Exper. M.*, N. Y., 1913, xvii, 71.

Ramirez, St. George and Moses. *J. Lab. & Clin. Med.*, St. Louis, 1924, x, 199.

Rappleye, W. C. *J. Biol. Chem.*, N. Y., 1918, xxxv, 509.

Rieger, J. B. *J. Lab. & Clin. Med.*, St. Louis, 1920, vi, 44.

Van Slyke, D. D., and Austin, J. H. *J. Biol. Chem.*, N. Y., 1920, xli, 345.

Van Slyke, D. D., and Donleavy, J. J. *J. Biol. Chem.*, N. Y., 1919, xxxvii, 551.

Whitehorn, J. C. *J. Biol. Chem.*, N. Y., 1921, xlv, 449.

CHAPTER VII

Blood Cholesterol

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Blood Cholesterol

(Modified Method of Bloor[17])

I. Solutions Used in the Determination of Blood Cholesterol

1. *Cholesterol Stock Solution*
   Cholesterol (Kahlbaum)..... 0.2 gm.
   Chloroform (pure)..... 200.0 c.c.
   1 c.c. = 1 mg. cholesterol.
2. *Cholesterol Standard Solution*
   Cholesterol stock solution..... 10.0 c.c.
   Chloroform (pure)..... 90.0 c.c.
   1 c.c. = 0.1 mg. cholesterol.
3. *Alcohol (redistilled)*
4. *Ether*
5. *Chloroform (dry)*
6. *Acetic anhydride*
7. *Sulphuric acid (concentrated)*

II. Preparation of Unknown Solution

Place 3 c.c. of whole blood slowly (with constant shaking of the flask) in a mixture of 60 c.c. of redistilled alcohol and 20 c.c. of ether in a 100 c.c. graduated flask. Shake thoroughly.

The flask is placed in a water bath on an electric hot plate and the contents carefully raised to boiling. Care should be taken not to overheat, by frequently shaking the flask. After boiling point has been reached, cool flask to room temperature, fill to the 100 c.c. mark with the alcohol-ether mixture, thor-

[17] In Bloor's method 1, 2 c.c. of sodium ethylate (3.0 gm. of metallic sodium dissolved in 100 c.c. of absolute alcohol) are added to each 10 c.c. of alcohol-ether extract.

In Bloor's method II, given above, the addition of sodium ethylate is omitted.

oughly mix and filter. (The filtered liquid will keep in a tightly stoppered dark bottle until the next day if necessary before completing the final determination.)

Place 10 c.c. of the alcohol-ether filtrate in a small beaker and evaporate just to dryness on a water bath or electric plate. Care should be used not to heat beyond the point of dryness, as a brownish color is produced, which renders the determination difficult.

The dry residue in the beakers is then extracted with successive small amounts (2 to 3 c.c.) of dry chloroform.[18] The residue and chloroform suspension are brought to a boil on a water bath. Decant each time after boiling to half volume into a 10 c.c. glass-stoppered graduated cylinder or graduated test-tubes. After cooling add chloroform up to 5 c.c. The solution should be colorless. Slight turbidity does not interfere. Add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid.[18] Mix by inverting cylinder several times. The unknown contains the equivalent of 0.3 c.c. blood.

III. Preparation of Standard Solution

Place 5 c.c. of the standard cholesterol solution in a 10 c.c. glass-stoppered graduated cylinder. Add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Mix by inverting cylinder several times. The standard solution which contains 0.5 mg. cholesterol begins to fade in about twenty minutes, so that comparison should be made within an interval of fifteen minutes.

IV. Final Step

Set tubes containing unknown and standard solutions aside at room temperature for about five minutes, after which place in

[18] Georgine Luden in her work on cholesterol found that any trace of water in the chloroform interfered with the subsequent color reaction. If the chloroform is kept in a wide glass bottle, into which has been placed a quantity of calcium chloride, any trace of water will be taken up by the latter substance. The chloroform should be filtered before use. Luden has found that adding 0.2 c.c. of concentrated sulphuric acid instead of 0.1 c.c., as generally recommended, produces a tone of green that can be matched more readily without interfering with the cholesterol values.

colorimeter cups for comparison with standard set at 15 mm. An average of three or four readings should be taken.

V. Calculation

$$\frac{15}{R} \times 0.5 \text{ mg.} = \text{mg. in } 0.3 \text{ c.c. blood,}$$

or

$$\frac{2500}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

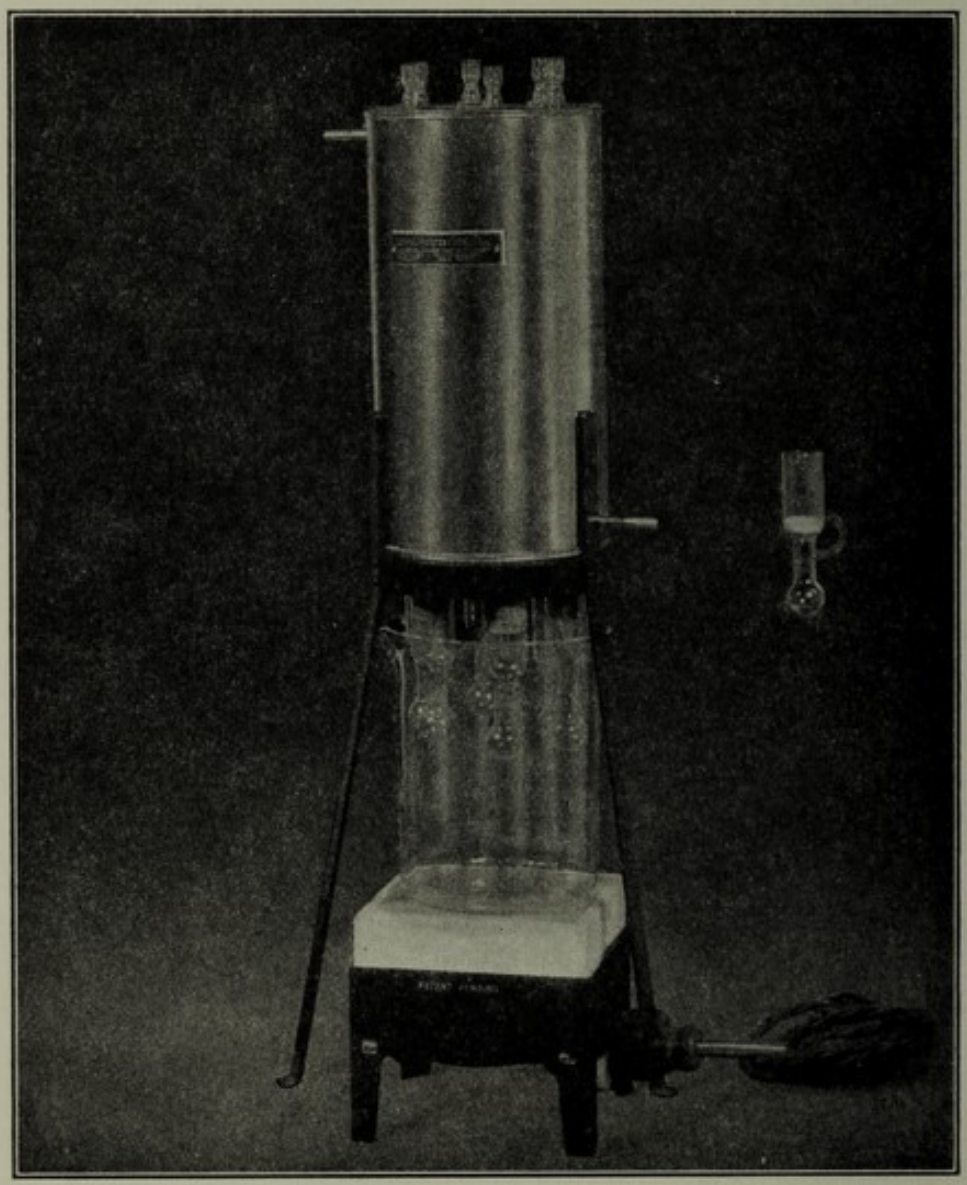

FIG. 7. Leiboff apparatus.

Blood Cholesterol

(Method of Leiboff)[19]

I. Solutions Used

1. Cholesterol Standard.
   Cholesterol, dry (Kahlbaum)..... 0.04 gm.
   Chloroform, dry, pure..... 500.0 c.c.
   5 c.c. = 0.4 mg. cholesterol.
2. Chloroform (dry) (see footnote [18]).
3. Acetic anhydride (pure, dry).
4. Sulphuric acid (concentrated).
5. Filter paper discs made from Whatman fat-free extraction thimbles. The discs have a diameter of ¾ inch and a thickness of 1/16 inch.
6. Extraction tube (see Fig. 7) marked at 5 c.c. It has neck attached to the side to permit passage of the chloroform vapors when used in the test.
7. Reflux condenser (Leiboff, see Fig. 7). The condenser is made of copper through which pass six glass tubes, thus allowing six simultaneous extractions. The glass tubes can easily be removed and replaced by new ones in case of breakage.

II. Preparation of Unknown Solution

About 5 c.c. of chloroform are put into the extraction tube. The chloroform level should reach a point about midway up the constricted portion of the tube. Twenty-five hundredths of a cubic centimeter of whole oxalated blood are placed by a pipette on the filter-paper disc, which absorbs it. If one filter-paper disc is insufficient to hold the blood, two or more discs may be used. The disc or discs are then dropped into the extraction tube and should rest at the upper part of the constricted portion of the tube. The tube is then attached to the reflux condenser and immersed in a beaker of hot or boiling

[19] This method is recommended because of its simplicity and accuracy. It is similar to the principle of extraction used in the method of Myers and Wardell.

water placed on a small electric stove. The level of the water in the beaker should be above the level of the chloroform in the tube. Extraction should be carried on for thirty minutes, after which the tube is detached from the condenser, the filter paper disc removed and the tube cooled by immersion in cool water for a minute or two. When cool add chloroform exactly to the 5 c.c. mark.

III. Preparation of Standard Solution

In a similar extraction tube place 5 c.c. of the standard cholesterol solution.

IV. Final Step

Add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid to each tube. Insert cork stoppers and invert tubes twice to mix well.

Place tubes in a beaker of cold water for half a minute to cool and leave in a dark place for ten minutes before reading in colorimeter.

V. Calculation

Set the standard at 10 or 15 mm., depending upon the intensity of color in the unknown. If the standard is set at 10 mm. the calculation will be as follows:

$$\frac{10}{R} \times 0.4 \text{ mg.} = \text{mg. in } 0.25 \text{ c.c. blood,}$$

or

$$\frac{1600}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

If the standard is set at 15 mm. the calculation will be as follows:

$$\frac{15}{R} \times 0.4 \text{ mg.} = \text{mg. in } 0.25 \text{ c.c. blood,}$$

or

$$\frac{2400}{R} = \text{mg. in } 100 \text{ c.c. blood.}$$

Clinical Comments on Blood Cholesterol

Cholesterol occurs in the blood as free cholesterol and in combination with fatty acids as cholesterol esters. *So far as known cholesterol cannot be synthesized or built up in the body.* It is believed that a balance exists in the body between the two blood lipoids, cholesterol and lecithin, but the factors governing this balance are unknown.

The origin of blood cholesterol is not definitely known. *Some of it arises from exogenous and some from endogenous sources.* It is subject to quite wide variations under conditions of disturbed metabolism. The test should not be regarded as a diagnostic test, but rather as a clinical test similar to the determination of hemoglobin or the test for albumin in the urine. The normal amount of cholesterol present in whole blood varies between 160 and 200 mg. per 100 c.c. In the plasma the figure is higher, averaging about 230 mg., while the corpuscle content averages about 200 mg. per 100 c.c.

In mild diabetes the cholesterol content of whole blood is usually increased to 240 to 250 mg. per 100 c.c., while in severe types of the disease the content is increased to 350 to 410 mg. per 100 c.c. The increase of the cholesterol content in diabetes is relatively proportionate to the increase of fat and total fatty acids of the blood in this disease, and has important prognostic significance. *The lower the cholesterol content of the blood, other things being equal, the longer is the expectation of life in diabetes.*

In cholelithiasis due to cholesterin concretions, the blood cholesterol is sometimes increased to 280 mg. or more, 950 mg. being mentioned by Hawk. This latter figure may be considered unusual, for many instances of proven cholelithiasis have not shown an increase in blood cholesterol above the range of normal values. Boyd has shown that the lipoids found in pathological gall-bladders are cholesterol esters. The esters occur in the plasma alone. From experiments upon dogs by Sweet and his associates it was found that the total blood cholesterol was increased to nearly double the normal immediately after removal of the gall-bladder. The normal level

was reached about forty days after operation and was considerably below normal after seventy-four days. From their work it appears that *free* cholesterol is not disturbed by fat ingestion or gall-bladder removal, while the rise in total cholesterol after removal of the gall-bladder was due to an increase in the amount of cholesterol *esters*.

The problems of disturbed cholesterol metabolism are concerned especially with the activity and quantity of bile and pancreatic juice available to convert cholesterol into esters. The adrenals and other glands are also concerned in the process. De Zani's experiments, mentioned by Georgine Luden in her work on cholesterol, have shown the importance of this lipoid to the cellular integrity of the organism. He fed mice on a cholesterin-free diet. During this time the animals drew upon their reserve deposits of cholesterol present in the body fat, brain, adrenal cortex and the liver, for cholesterol was present in the feces. The mice increased in size and weight, but they all died at the end of seventeen days. An increase of blood cholesterol occurs in pregnancy after the fourth month and persists for a considerable period after term, in nephritis, in arteriosclerosis, in obstructive jaundice and in the early stages of malignant neoplasms. In hemolytic icterus the blood cholesterol is not increased.

The work of Epstein and Lande has called attention to the importance of blood cholesterol studies in connection with protein deficiency and decreased basal metabolism, especially in parenchymatous nephritis (nephrosis). In their observations on 6 such patients the basal metabolism was subnormal and was associated with high blood cholesterol figures. In these patients with large amounts of albumin in the urine and decreased output associated with extensive edema, and who did not improve upon salt-free, Karell and carbohydrate diets, an improvement in basal metabolism and decreased edema was noted under a high protein diet together with the administration of thyroid extract. In myxedema, which may be associated with nephrosis, the blood cholesterol may be increased. Under thyroid therapy, with improvement in the basal metabolic rate, in such cases the blood cholesterol has

decreased. In 2 patients with nephrosis, recently observed, the blood cholesterol was increased above 300 mg. per 100 c.c. and the basal metabolic rate was below the normal limits. The result secured in one of these patients was striking when a high protein diet (without eggs) was given together with thyroid extract. Complete recovery occurred in this patient, who had been bedridden with anasarca for over six months. In a more recently observed patient with nephrosis the blood cholesterol was increased to 588 mg. per 100 c.c. His basal metabolic rate was minus 18.9 per cent. Campannaci has also called attention to the benefit obtained from thyroid administration in 3 patients with nephrosis. Eppinger first emphasized the relationship existing between the thyroid gland and kidney function, and the influence of thyroid extract upon diuresis and edema as well as the mobilization of salt and the utilization of protein.

In exophthalmic goiter with marked increase in the basal metabolic rate, as well as in toxic adenomas of the thyroid, the blood cholesterol values are usually lower than normal. The following table arranged from the publication of Epstein and Lande recapitulates their findings:

BLOOD CHOLESTEROL CONTENT IN RELATION TO METABOLIC RATE

Condition	Number Observations	Basal Metabolic Rate	Average Cholesterol in Mg. Per 100 c.c. Blood	Method
Parenchymatous nephritis (nephrosis).	6	subnormal	510	Bloor modification of Funk-Autenreith method.
Chronic diffuse nephritis.	4	subnormal	300	
Myxedema.....	1	−19%	1350 (decreased to 206 under thyroid therapy)	Bloor modification of Funk-Autenreith method.
Myxedema.....	1	−14%	313	
Non-toxic adenoma of thyroid.	6	normal	176	Bloor modification of Funk-Autenreith method.
Menopause.....	10	normal	234	
Exophthalmic goiter.	21	+44%	144	Bloor modification of Funk-Autenreith method.
Toxic adenoma of thyroid.	10	+29%	182	

Since a large reserve of cholesterol is present in the cells of the body and since practically all staple foods contain it in varying amounts, it is not likely that a marked deficit occurs in the human body except under conditions of starvation, in the presence of wasting diseases such as carcinoma and Addison's disease, the fatal cases of exophthalmic goiter and the severe anemias.

The diet most suitable for patients with excessive amounts of blood cholesterol but with normal metabolic rates, should consist of fruit, vegetables and milk, eliminating largely those foods which are known to contain high cholesterol values, such as eggs, especially egg yolk, fish roe, fat fish, bacon and fat meats, butter, cream, creamed soups, cheese (except cottage cheese), olive oil or table oils, and nuts, since with normal metabolic rates there probably will be no deficiency in protein utilization. An excess of carbohydrates in the diet calling for excess work on the part of the pancreas may under certain conditions prevent proper esterification of the blood cholesterol and its elimination, with resulting concentration of cholesterol in the blood. This was observed by Luden in her experimental oatmeal diet.

For patients, however, who manifest disturbances associated with subnormal metabolic rates and increased blood cholesterol, the combination of high protein diet and thyroid therapy should be tried on the basis that the possible benefit from the administration of thyroid stimulates the rate of oxidation and promotes the assimilation of protein, the utilization of which is impaired. As mentioned by Epstein and Lande, the effect of thyroid in promoting utilization of protein may explain its influence on edema, especially in parenchymatous nephritis.

Rothschild and Rosenthal have advocated diets with low cholesterol values in the treatment of cholelithiasis associated with increased blood cholesterol. Wilensky and Rothschild have recently shown that among 115 patients with gall-bladder disease, postoperative symptoms occurred in 26 per cent. Of these about 10 per cent required reoperation for complications which were purely surgical, such as fistula, stone, cholangitis

or obstructive jaundice. The cause of the postoperative symptoms in 16.5 per cent was, in their opinion, purely a medical problem due to the underlying disturbance of the cholesterin metabolism which produced or was associated with hypercholesterinemia. In such cases the blood cholesterol was increased to 250–300 mg. per 100 c.c. The postoperative symptoms varied from a dull ache referred to the liver region with flatulency and constipation, to severe cramps which usually subsided spontaneously. In others severe biliary colic with vomiting and varying degrees of jaundice occurred. The judgment of the clinician may be sorely taxed by such symptoms and the tendency may be strong in favor of reoperation. Medical measures, such as free purgation and a dietary régime which restricts the fatty foods, have been found to relieve the symptoms in a large proportion of such patients and are worth the effort even though the suspicion may be well grounded that common duct obstruction or new stone formation has occurred.

The experimental work of McMaster has brought evidence to show that the output and concentration of cholesterol in the bile are markedly influenced by the diet and that such effects are independent of any elaboration by the gall-bladder itself.

References on Blood Lipoids

Bloor, W. R. *J. Biol. Chem.*, N. Y., 1914, xvii, 377; *ibid.*, 1915, xxiii, 317; *ibid.*, 1916, xxiv, 227, xxv, 577; *ibid.*, 1917, xxix, 437.

Bloor, W. R., and Knudson, A. *J. Biol. Chem.*, N. Y., 1917, xxix, 7.

Bloor, W. R., and McPherson, D. *J. Biol. Chem.*, N. Y., 1917, xxxi, 79.

Boyd, W. *Brit. J. Surg.*, Bristol, 1923, x, 337.

Campanacci, D. *Wien. klin. Wchnschr.*, 1924, xxxvii, 257.

Chauffard, Laroche and Grigaut. *Compt. rend. Soc. de biol.*, Paris, 1911, lxx, 70, 536.

Chauffard, Richet and Grigaut. *Compt. rend. Soc. de biol.*, Paris, 1911, lxx, 276.

Epstein, A. A., and Lande, H. *Arch. Int. Med.*, Chicago, 1922, xxx, 563.

Gray, H. *Boston M. & S. J.*, 1918, clxxviii, 16, 50, 91, 120, 156. *Am. J. M. Sc.*, Phila., 1924, clxviii, 35.

Hawk, P. B. Pract. Physiol. Chem. Ed. 8, Phila., 1923.

Henes, E., *Arch. Int. Med.*, Chicago, 1920, xxv, 411. *Deutsch. Arch. f. klin. Med.*, 1913, cxi, 122.

Knudson, A. *J. Biol. Chem.*, N. Y., 1921, xlv, 255.

Leiboff, S. L. *J. Biol. Chem.*, N. Y., 1924, lxi, 177; *J. Lab. & Clin. Med.*, St. Louis, 1925, x, 857.

LUDEN, G. *J. Lab. & Clin. M.*, St. Louis, 1916, i, 662; *ibid*, 1917, iii, 141; *ibid.*, 1918, iv, 849; *ibid.*, 1919, iv, 719. *J. Biol. Chem.*, N. Y., 1916, xxvii, 273.
MCMASTER, P. D. *J. Exper. M.*, N. Y., 1924, xl, 25.
MÖNCKEBERG, J. G. *Berlin klin. Wchnschr.*, 1924, iii, 1521.
MYERS, V. C., and WARDELL, E. L. *J. Biol. Chem.*, N. Y., 1918, xxxvi, 147.
RANDLES, F. S., and KNUDSON, A. *Science*, N. Y. and Lancaster, 1924, lx, 530.
RÉMOND and ROUZAUD. *Bull. Acad. de méd.*, Paris, 1923, lxxxix, 60.
ROTHSCHILD, M. A., and ROSENTHAL, N. *Am. J. M. Sc.*, Phila., 1916, cli, 394.
ROTHSCHILD, M. A., and WILENSKY, A. O. *Am. J. M. Sc.*, Phila., 1918, clvi, 239, 404, 564.
SCHMIDT, H. B. *Arch. Int. Med.*, Chicago, 1914, xiii, 121. *Ann. Clin. Med.*, 1922, i, 66.
STRAUSS, H., and SCHUBARDT, W. *Zentralbl. f. innere. Med.*, Leipz., 1922, xliii, 425.
SWEET, J. E. *Internat. Clin.*, Phila., 1924, i, 187.
WESTON, P., and KENT, G. *J. Med. Research*, Bost., 1912, xxvi, 531.
WILENSKY, A. O., and ROTHSCHILD, M. A. *Am. J. M. Sc.*, Phila., 1924, clxviii, 66.

CHAPTER VIII

Total Nitrogen Determination in the Urine
Titratable Acidity of the Urine
Phenolsulphonephthalein Determination

CHAPTER VIII

Total Nitrogen Determination in the Urine

(Modified from the Method of Folin)

1. With a measuring pipette place 0.2 c.c. filtered urine and 0.5 c.c. of distilled water in a Pyrex ignition test-tube. With an ordinary pipette add 0.5 c.c. of the phosphoric-sulphuric-acid digestion mixture, made as follows:

Acid Phosphoric-Sulphuric Digestion Mixture

Copper sulphate solution (5 per cent)	50.0 c.c.
Acid phosphoric (85 per cent)	300.0 c.c.
Acid sulphuric, c. p. (free from ammonia)..	100.0 c.c.
Distilled water	450.0 c.c.

This should be kept well stoppered to prevent absorption of ammonia from the air. (Same as Solution 1 for blood non-protein nitrogen determination.)

2. Heat slowly over a micro-burner until the water is driven off and the color changes to dark brown, then cover tube with watch glass and continue heating gently until dense fumes fill the tube. Continue boiling at such a rate that the tube contains fumes but almost no fumes escape. The color should become clear or bluish-green after partially cooling. A drop of hydrogen peroxide may be added to clear the solution while the tube is warm. Remove flame and let cool for about two minutes. Rinse the tube thoroughly (the contents may be turbid from silica) with a little distilled water into a 25 c.c. volumetric flask. Shake to mix.

3. Transfer with a measuring pipette 1 c.c. of standard nitrogen solution (used in the determination of non-protein nitrogen in the blood, and which contains 0.2 mg. N per c.c.) into a 50 c.c. volumetric flask, add 1 c.c. of the phosphoric-sulphuric acid mixture to balance the acid in the unknown,

add about 10 c.c. of distilled water and shake to mix. The standard so prepared contains 0.2 mg. nitrogen.

4. When ready give each flask a whirl and add about 10 c.c. of Nessler's reagent to the unknown, and about 20 c.c. to the standard. When full development of color has been secured add sufficient distilled water to bring volume in the unknown to the 25 c.c. mark and in the standard to the 50 c.c. mark.

5. If the unknown nesslerized mixture is turbid from silica, centrifuge a portion before the color comparison is made. The white sediment in the tube is silica. *If the sediment is deeply colored the nesslerization was not successful and should be discarded.* Wait five minutes for color to develop before comparing in the colorimeter.

6. With standard set at 20 mm., with R indicating the reading of the unknown solution, the calculation will be as follows:

$$\frac{20}{R} \times \frac{0.2 \text{ mg.}}{2} = \text{mg. N in 0.2 c.c. urine,}$$

or

$$\frac{1000}{R} = \text{mg. N in 100 c.c. urine.}$$

7. Example: If the twenty-four hour quantity of urine equaled 1560 c.c. and the reading of the unknown was 15, the result would be $\frac{1000}{15} \times 15.6 = 1040$ mg. or 1.04 gm.

Titratable Acidity of Urine, in Terms of N/10 NaOH (Method of Folin)

1. Place 10 c.c. of urine in a flask, add about 6 gm. of finely pulverized neutral potassium oxalate,[20] and 1 to 2 drops of 1 per cent phenolphthalein solution as indicator.

2. Shake solution vigorously one to two minutes and titrate with N/10 NaOH until the solution turns a faint pink which remains permanent when the solution is shaken.

3. If 1200 c.c. represents the twenty-four hour volume of urine, and 6 represents the number of cubic centimeters of N/10 NaOH used, the total acidity will be calculated as follows:

$10:6::1200:x$ or, $10x = 7200$ or, $x = 720$, the acidity of the twenty-four hour urine in terms of cubic centimeters of N/10 NaOH.

The acidity may also be represented in terms of percentage. In the example above the acidity per 100 c.c. in terms of N/10 NaOH would be 60.

[20] Potassium oxalate is added to precipitate the calcium which would interfere with the titration end-point when the urine is neutralized.

Phenolsulphonephthalein Determination of Kidney Function

1. Have patient empty bladder and then drink 300–400 c.c. of water.

2. Twenty minutes later inject 1 c.c. (from ampule containing 6 mg.) of phenolsulphonephthalein into gluteal or lumbar muscles.

3. One hour and ten minutes later have patient empty bladder (ten minutes of this time interval is to allow the dye to reach the kidneys). Add 200 c.c. water to urine and 1 c.c. of 10 per cent sodium hydroxide solution to bring out the deep purple color, then dilute to 1000 c.c. Label "first-hour" specimen.

4. One hour later have patient empty bladder. Add 200 c.c. of water to the urine and 1 c.c. of 10 per cent sodium hydroxide solution, then dilute to 1000 c.c. Label "second-hour" specimen.

5. The standard solution required contains 3 mg. phenolsulphonephthalein and 1 c.c. of 10 per cent sodium hydroxide solution and volume made to 1000 c.c.

6. Set standard in colorimeter at 10 and make comparison with first- and second-hour specimens. R equals reading of unknown. The calculation will be $\frac{10}{R} \times 50$ or $\frac{500}{R}$ = per cent of dye excreted.

7. The first-hour specimen should equal 40–60 per cent; the second-hour specimen 20–25 per cent more, or a total in the two-hour period of 60–85 per cent. *An average of many so-called normal estimations has equaled 48 per cent for the first hour and 17 per cent for the second hour, or a total of 65 per cent.*

CHAPTER IX

Clinical Comments on the Diagnosis of Impaired Kidney Function

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Clinical Comments on the Diagnosis of Impaired Kidney Function

Degenerative processes affecting the cardiovascular renal systems, produced by many processes, including acute and chronic infections as well as the wear and tear incidental to life, are such important factors leading to death before the expiration of the normal life expectancy, and constitute such an important part of the work of the physician in his endeavor to prevent the development of such lesions, that an understanding of this phase of clinical medicine is essential in the care of practically every patient who has reached the fifth decade of life.

The endeavor to determine what factors are concerned in producing evidence of early damage to these organs involves in practically every case knowledge of the blood chemistry as well as an investigation of kidney function in a more comprehensive manner than such tests are usually performed. A little time and a comparatively small outlay spent in equipping a laboratory, as well as training a young woman in the essentials of the work, will make available for every physician the information desired. The reward in added satisfaction that the advice given is founded upon fact, rather than upon the basis of a casual examination of a single urinary specimen, will be many times worth the time consumed and the effort used. Among the essential facts to be determined in an examination of kidney function are the following:

The Urine Examination

1. In the absence of edema the quantity of urine voided varies normally with the intake of fluid. Variations in humidity, the amount of physical exercise, and the occurrence of diarrhea may be mentioned as factors disturbing the normal ratio of

intake to output. Normally the quantity of urine voided during the twelve-hour night period should not exceed 50 per cent of the quantity voided during the twelve-hour day period.

2. The specific gravity should vary between 1.010 and 1.030, depending upon the amount of fluid taken, the humidity and other factors, such as exercise and nervous or emotional stress. Normally there should be evident no tendency toward so-called fixation of specific gravity toward either the higher or the lower points in a series of specimens passed at different periods during the twenty-four hours.

3. All specimens should normally be free from abnormal ingredients such as albumin, sugar, diacetic acid, casts, blood-cells and pus. A few leucocytes in specimens from patients who have suffered a previous inflammatory or traumatic lesion in any portion of the genitourinary tract may be found for long periods subsequently. Specimens from women who have borne children and who have relaxed bladder walls normally contain a considerable number of leucocytes. The finding of an occasional hyaline cast in a centrifugalized specimen, especially from an individual near the middle point of life, may be ignored.

4. The urea content of any specimen should be 2 per cent or higher. The hypobromite method for urea is sufficiently accurate for clinical purposes. If the patient has been under supervision with weighed diets so that the total intake of protein in grams is known (from which the nitrogen intake can be computed by dividing by 6.25) it aids in an understanding of the patient's condition if the "nitrogen balance" is determined by a computation of the total nitrogen eliminated in the twenty-four hour specimen of urine.

5. The output of sodium chloride in any specimen varies according to intake from 1 to 2.5 per cent or higher. The Volhard method is satisfactory. A modification of this method may be performed as follows: Dilute 10 c.c. of urine with 90 c.c. of water to which should be added 1 or 2 drops of 25 per cent nitric acid. The mixture should then be made alkaline with 10 per cent solution of sodium carbonate. A few drops of

a 10 per cent solution of potassium chromate solution are added as an indicator. Titration is then performed with N/10 silver chloride solution. Each cubic centimeter of the silver solution used equals 0.00583 gm. of sodium chloride.

6. The output of phthalein as a measure of the excretory function of the kidneys should under normal conditions approximate 40 to 60 per cent during the first hour, and 20 to 25 per cent during the second hour, or a total of 60 to 85 per cent during the first two hours.

7. The usual tests for acetone bodies in the urine are, as Folin has shown, tests for diacetic acid. The sodium nitroprusside test is more delicate than the ferric chloride reaction. These tests, when persistently positive, indicate disturbed metabolism dependent usually upon dehydration and so-called "acid poisoning." The administration of salicylates and coal-tar products such as phenacetine may produce a diacetic reaction in the urine.

The Blood Examination

1. The test diet of Mosenthal designed to measure the excretory capacity of the kidneys has served a useful purpose in calling attention to phases of the subject not generally appreciated. For its proper interpretation most careful attention to detail is necessary, conditions being obtained, as a rule, only with the cooperation of a trained dietician during the period of observation. Under this plan blood-chemistry studies and deductions incidental to the test diet and measured fluid intake are correlated with examinations of the measured urinary specimens, all of which has for its purpose the determination of kidney function under more or less artificial conditions. There are many reasons for believing, as Mosenthal has stated,[21] *that if the various phases of renal function are studied while the patient is following his usual daily routine, a more valuable estimate of how he should adjust his habits to the conditions found to exist may be obtained than by carrying out artificial dietetic tests that*

[21] Mosenthal, Herman O. Value of tests for renal function in clinical medicine. *Ohio M. J.*, Columbus, May, 1922.

impose standards which may not be applicable or approximate the normal daily routine of the individual.

It is obvious that in carrying out any investigation the ordeal must be simplified as much as possible for the patient. For ambulatory patients the following plan is recommended: For the twenty-four hours preceding the time set for taking the sample of blood before breakfast, the patient collects the urine specimens as follows: Upon arising the bladder is emptied. This urine is discarded. All specimens passed from 8 a.m. to and including 8 p.m. are measured and a portion of each placed in bottles which are to be brought to the laboratory. The urine passed after 8 p.m. to 8 a.m., including the amount voided upon arising, is collected in one container, measured, and a portion labeled "8 p.m. to 8 a.m." is brought to the laboratory. The patient is instructed to drink no fluid after the evening meal. If possible a record should be kept of the amounts of fluid taken during the day. For many patients it is only necessary to collect and measure the urine passed during the day and night twelve-hour periods, a portion of each being brought for examination. The data accumulated in such an examination may conveniently be summarized in the form of a chart which includes the blood-chemistry findings.

		Day quantity After 8 a.m.–8 p.m.	Night quantity After 8 p.m.–8 a.m.
Urine Specimens *Quantity c.c.*			
Specific gravity of different specimens.			
Greatest variation in specific gravity	=		
Total nitrogen output 24-hour quantity	=		
Total sodium chloride 24-hour quantity	=		
Abnormal ingredients, Albumin	=		
Sugar	=		
Diacetic acid	=		
Chlorides	=		
Microscopic			
Phthalein output, first hour	=	per cent; second hour =	per cent
Fasting blood chemistry		mg. per 100 c.c. blood.	
Non-protein nitrogen	=		
Urea	=		
Preformed creatinin	=		
Uric acid	=		
Sugar	=		
Chlorides	=		
Cholesterol	=		

CHAPTER X

The Dietary Control of Disturbances of Metabolism

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The Dietary Control of Disturbances of Metabolism

I. Neutral, Alkali and Acid-Producing Foods

The following lists of foods useful in the treatment of certain disturbed metabolic states are appended. They are based upon the ash analyses of Sherman and Gettler and have been tested on man by Blatherwick.The first three have been published by Sansum of the Potter Metabolic Clinic.

1. *Neutral Foods*

Butter	Cornstarch
Cream	Sugar
Lard	Tapioca

2. *Alkali-Producing Foods*

In the following list the excess of base or alkali over acid is expressed in terms of cubic centimeters of a normal solution.

	Per 100 Gm.
FRUITS	
Raisins	23.68
Muskmelons	7.47
Pears, dried	7.07
Currants	5.97
Oranges	5.61
Bananas	5.56
Lemons	5.45
Peaches	5.04
Apples	3.76
VEGETABLES	
Beans, lima, dried	41.65
Beans, dried	23.87
Beets	10.86

VEGETABLES (Cont.)

	Per 100 Gm.
Carrots	10.82
Celery	7.78
Lettuce	7.37
Potatoes	7.19
Peas, dried	7.07
Cauliflower	5.33
Cabbage	4.34
Radishes	2.87
Turnips	2.68
Asparagus	0.81
Tomato	

NUTS

Almonds	12.38
Chestnuts	7.42

MISCELLANEOUS

Cow's milk	2.37

3. *Acid-Producing Foods*

In the following list the total excess of acidity over base is expressed in terms of cubic centimeters of a normal solution. The ash of these foods is alkaline in reaction, but since they contain glycinformers that assist in the formation of benzoic acid, which is changed to hippuric acid before elimination, the body acidity is increased when these foods preponderate in the diet.

MEATS AND FISH	Per 100 Gm.
Oysters	30.0
Chicken	17.0
Haddock	16.0
Venison	15.8
Rabbit	14.8
Beef, lean	13.9
Veal	13.5
Pike	11.8
Pork, lean	11.8
Frog	10.3

EGGS	
Yolk	26.6
Eggs, whole	11.1

CEREALS	
Oatmeal	12.9
Rice	8.1
Bread,[22] white or whole wheat	7.0
Crackers	7.8
Corn, sweet, dried	5.9

[22] Sherman. Food Products. 1914 edition.

NUTS

	Per. 100 Gm
Peanuts	3.9

FRUITS

Cranberries	
Prunes	
Plums	

For reference the following table has also been found useful. In it the excess of base or alkali over acid in 100 calories of each food is given:

Spinach	113.0	Buttermilk	6.1
Cucumbers	45.5	Squash	6.1
Celery	42.1	Apples (fresh)	6.0
Chard	41.1	Pumpkin	5.7
Lettuce	38.6	Bananas	5.6
Rhubarb	37.0	Pears (fresh)	5.6
Figs	32.3	Potatoes (sweet)	5.4
Tomatoes (canned)	24.5	Milk (skimmed)	5.0
Carrots	24.0	Beans (baked)	5.0
Beets (fresh)	23.6	Grape-juice	4.0
Molasses	20.0	Potatoes (chips)	3.9
Muskmelons	19.0	Asparagus	3.6
Olives	18.8	Chestnuts	3.2
Parsnips	18.2	Dates	3.2
Cabbage	18.0	Onions	3.1
Cauliflower	17.4	Citron	3.0
Pineapple	15.7	Koumiss	2.9
Orange-juice	14.4	Grapes	2.8
Beans (string)	13.0	Milk (condens. unsw.)	2.7
Raspberry juice	13.0	Milk (whole)	2.6
Peaches (fresh)	12.2	Beans (kidney)	2.5
Lemons	12.0	Pears (canned)	2.3
Oranges	11.0	Almonds	1.8
Lemon-juice	11.0	Currants	1.8
Apricots	11.0	Peas (canned)	1.5
Peaches (canned)	10.0	Peas (dried)	1.5
Radishes	9.8	Milk (condens. sweet)	1.4
Mushrooms	8.9	Cocoanuts	1.2
Watermelon	8.8	Peas (green)	1.2
Potatoes (white)	8.6	Cream (18% fat)	0.3
Cherries	7.8	Cream (40% fat)	0.1
Turnips	7.0	Marmalade	0.1
Raisins	6.8	Cocoa	0.1

II. Minimum Salt and Basic Alkali Diet

For general purposes the following list of foods has been useful in the treatment of edema in chronic nephritis of the types associated with hyperarterial tension. In this list the content of salt is small, the protein restricted and an excess of alkali-producing foods is present in the amounts ordinarily consumed. The amount of fluid intake should be restricted to 700–1000 c.c. daily. The following foods are allowed: Bread or toast (made without salt), potato, sugar, farina, custard, fresh butter, rice, tea, milk (250 c.c. daily), baked apples or apple sauce, orange juice, melons, grapefruit, lettuce, jello, bananas, pears, peaches, figs and pineapple. In general all fruits may be allowed except cranberries, prunes and plums. All thoroughly cooked green vegetables may be allowed.

Type of Basic-Alkali Diet

(Low protein and low purine)

Breakfast

One glass orange juice[23]
Baked apple with cream (or stewed fruit)[24]
Two slices bacon (or portion cream of wheat)
One-half slice white toast. Butter, jelly or marmalade
Cup hot milk with coffee to flavor or Kaffee Hag

Luncheon

White or sweet potato in any form
Vegetables[25]
Lettuce, vegetable or fruit salad (French dressing one part lemon juice and four parts salad oil)
One-half slice white bread
Butter. Ripe olives

[23] The orange juice greatly assists in rendering the urine alkaline.

[24] Avoid plums and prunes.

[25] The following vegetables may be used: Beets, carrots, celery, turnips, cauliflower, asparagus, spinach, rhubarb, cabbage, string beans, squash, tomatoes, peas, parsnips, cucumbers, onions.

Melon or stewed fruits
One glass orange juice (diluted grape or loganberry juice or buttermilk)

Dinner

Any vegetable soup
White or sweet potato in any form
Vegetables
Lettuce, vegetable or fruit salad (French dressing)
One-half slice white bread. Butter
One glass orange juice
Nuts (almonds or chestnuts)
Raisins (figs or dates)
Stewed fruit (or banana, apricot, pineapple, cocoanut or apple pie)
Ice cream, fruit ice, fresh grapes, jello

III. Clinical Comments

The accumulated experience of many physicians has shown that patients who have disturbed kidney function, definite nephritis, hyperarterial tension, rheumatic symptoms (when due to improper diet), headaches due to overfeeding, lack of exercise and improper elimination, obscure neuralgic and neuritis-like pains (not due to foci of chronic infection) and other similar conditions, are generally benefited by restriction of the meats and eggs. This benefit aside from the restricted albumin intake may with much reason be ascribed, especially in most typical types of chronic nephritis and the associated acidosis, to the influence of diminished acid formation, the deleterious effect of which upon the kidneys has been thoroughly investigated by Martin Fischer. The only apparent exceptions to such a régime as regards the protein intake are to be found in the treatment of certain types of chronic nephritis in which subnormal basal metabolism may be present and in which it may

be believed that impaired utilization of protein exists. This has been discussed under blood cholesterol.

It will be noted from the above list that certain cereals are acid-producing foods. From a practical standpoint, if the diet is largely made up of the base or alkali-producing foods given above, the moderate consumption of breadstuffs will not greatly influence the result desired.

In general, with the exception of cranberries, prunes and plums, all fruits, vegetables and nuts (with the exception of peanuts) are basic (alkaline) in nature. The following foods, among those mentioned in the list above, have been found useful clinically in reducing the body acidity as measured by the reaction of the urine: Melons, apples, oranges, bananas, lemons, carrots, beets, lettuce, celery and potatoes. The fruit acids are largely converted into alkali carbonates in the intestine, hence they may be considered alkaline in nature. The neutral foods do not increase the body acidity and may be used in ordinary amounts unless special reason for restriction, such as acidosis, exists, in which case the intake of fats, such as cream, butter and lard, should be limited. For ordinary clinical control I have found it useful to give to the patient or nurse a supply of methyl-red papers, after a suggestion by Martin Fischer, by means of which the reaction of the urine may be tested once or twice daily.

IV. The Purine Constituents of Foods

The purine constituents of foods contain the chemical compound C_5N_4. The most important purine compound is uric acid, but xanthine, hypoxanthine, caffeine, theine, guanine, adenine and theobromine are included in this group.

Since it appears highly desirable to limit the intake of purine-containing foods in the treatment of conditions due to abnormal retention of uric acid in the blood, the following table of Walker Hall is added:

I

Meats	*Purine bodies, gm. per kilo.*
Thymus (sweet bread)	10.0
Liver	2.75
Beef	1.3–2.0
Pork	1.2
Chicken	1.2
Ham	1.1
Veal	1.1
Salmon	1.1
Halibut	1.0
Mutton	0.96
Cod	0.5
Meat soups	(varying large amounts)

Vegetables	*Purine bodies, gm. per kilo.*	*Drinks*[26]	*Per 500 c.c.*
Beans, kidney	0.63	Coffee	1.7
Oatmeal	0.53	Tea	1.2
Peas	0.39	Cocoa	1.0
Lentils	0.38	Chocolate	0.7
Asparagus	0.21		

The foods listed in the above table should be limited in conditions associated with abnormal uric-acid retention. *In addition to those mentioned, the glandular meats, such as kidney and brain; whole-wheat products, such as graham or whole-wheat bread or shredded wheat; and malt preparations, such as beer, ale or porter, should be restricted.*

The following foods are purine-free or contain a negligible amount[27]:

Cereals	*Vegetables*	
Rice	Potatoes (Irish or sweet)	Eggplant
Hominy	Cauliflower	Spinach
Farina	Onions	Brussels sprouts
Cream of Wheat	Cabbage	Corn
Cornflakes	Lettuce	

[26] The methyl purines present in coffee, tea and chocolate are not as a rule rigorously excluded in the diet. A caffeine-free coffee, such as Kaffee Hag, may be allowed.

[27] From von Noorden and the Vanderbilt Clinic Diet Lists.

Breadstuffs

Flour (white)
Bread (white)
Corn meal
Spaghetti
Macaroni
Biscuits (white)
Crackers (white)

Dairy Products

Butter
Milk
Buttermilk
Cream

Desserts

Nuts
Cheese (American, Swiss, and Cream)
Ice cream and ices
Cake (except coffee or chocolate)
Puddings (bread, tapioca, or cornstarch)
Pie (apple, custard, or cocoanut)
Jello

Miscellaneous

Jam and marmalade
Sugar and syrup
Fresh and cooked fruits
Bacon
Soups (cream or vegetable)
Eggs

Beverages

Carbonated water
Vichy
Grape-juice
Loganberry-juice
Cider
Malted milk

The following "moderate protein-low purine" diet list is given which consists of neutral and alkali-producing foods useful in the treatment of nephritis. It contains approximately 2000 calories, an amount sufficient to maintain efficiency for the ambulatory patient who is obliged to do a moderate amount of work. For obese patients a diet may be appropriately followed which consists largely of fruits and vegetables.

Moderate Protein-Low Purine Nephritic Diet

	Wt. in gm. or c.c.	Protein	Fat	Carbohydrate	Calories	Remarks
Breakfast						
1 egg	...	6.2	5.6	....	76.0	
Farina (cooked)	100	1.7	0.2	11.5	56.0	If 100 gm. hominy are used prot. = 2.2; fat = 0.2; carbo. 17.8; calories 84. If 100 gm. boiled rice, prot. 2.8; fat 0.1; carbo. 24.4; calories 112
Sugar	15	....	.....	15.0	60.0	
Cream (20 per cent)	90	3.0	18.0	3.0	180.0	Usual market cream
Toast (white bread 2 slices)	60	5.5	0.8	32.0	160.0	
Butter	15	0.15	12.7	....	119.0	If edema is present use fresh butter
Orange or peach marmalade (1 hp. tablespoonful)	30	0.18	.....	25.3	105.0	
Luncheon						
Cream vegetable soup	250	6.4	18.0	14.6	242.0	Made without meat stock from corn, potatoes, asparagus, celery, cauliflower, thickened with cornstarch
Butter	15	0.15	12.7	....	119.0	
Bread (white 2 slices)	60	5.5	0.8	32.0	160.0	If edema is present use bread made without salt
Dinner						
Vegetables (cooked)	60	1.0	.....	2.0	12.0	Vegetables with 3 per cent available carbohydrate used, which include the following: Asparagus, cauliflower, Brussels sprouts, beet greens, cabbage, rhubarb, spinach, string beans, eggplant. If vegetables with 6 per cent available carbohydrate are used, such as the following: Onions, squash, turnips, carrots, mushrooms, or beets, add 1.0 gm. protein and 2.0 gm. carbohydrate to intake and 12 calories
Potato (baked) med. size	130	3.7	0.2	32.0	145.0	
Milk (glass)	180	6.0	7.2	9.0	130.0	
Head lettuce (½ head)	50	0.6	0.15	1.5	10.0	
French dressing (1 tbsp.)	22	....	16.0	....	150.0	Made in proportion 4 tbsp. olive or other vegetable oil, 1 tbsp. lemon juice, ¼ teasp. salt
Bread (white 1 slice)	30	2.75	0.4	16.0	80.0	
Butter	15	0.15	12.7	....	119.0	
Baked apple	120	0.6	0.6	30.0	128.0	3 heap. tbsp. apple sauce contains prot. 0.25; fat 1.0; carbo. 46, calories 194
Totals	...	43.5	106.0	224.0	2051.0	

References on the Acid, Alkali and Purine Constituents of Foods

Blatherwick, N. R. *Arch. Int. Med.*, Chicago, 1914, xiv, 409.
Blatherwick, N. R., and Long, M. L. *J. Biol. Chem.*, N. Y., 1922, liii, 103; *ibid.*, 1923, lvii, 815.
Fischer, M. Nephritis. N. Y., 1912. *J. A. M. A.*, Chicago, 1913, lx, 1682.
Hall, J. W. The Purine Bodies of Food Stuffs, Lond., 1903.
Henderson, L. J., and Palmer, W. W., *J. Biol. Chem.*, N. Y., 1913, xiii, 393.
Sansum, W. D., Blatherwick, N. R., and Smith, F. H. *J. A. M. A.*, Chicago, 1923, lxxxi, 883.
Sherman, H. C., and Gettler, A. O. *J. Biol. Chem.*, N. Y., 1912, xi, 323.

CHAPTER XI

Brief Comments on the Diet in the Treatment of Diabetes Mellitus

CHAPTER XI

Brief Comments on the Diet in Diabetes Mellitus

In order to have them available for ready reference the following tables of food proportions found useful in the treatment of diabetes mellitus are appended. The principles of dietary management are based upon the experience of many clinicians and upon the work of many laboratory workers. It is taken as established that the concensus of opinion favors a minimum of 1 gm. of protein per kilo for adults as the proper amount to preserve nitrogen equilibrium. It is also taken as established that a minimum of 30 calories per kilo for adults is required for basal maintenance at rest. It is also believed that a proper relationship should exist in the diabetic diet between the acid-forming or ketogenic constituents and the antiacid or antiketogenic constituents. The ketogenic elements are 90 per cent of the fat and 46 per cent of the protein constituents. The antiketogenic elements are 10 per cent of the fat, 58 per cent of the protein, and 100 per cent of the carbohydrate constituents. The formula in which the number of grams of each constituent is known may be expressed as follows:

$$\frac{\text{Fatty acids (ketogenic)}}{\text{Glucose (antiketogenic)}} = \frac{0.9F + 0.46P}{0.1F + 0.58P + C}$$

Experience has shown, following the publications of Shaffer and of Woodyatt, *that a diet in which the ratio of the ketogenic to the antiketogenic elements does not exceed 1.5 to 1.0 renders diabetic patients relatively safe from the dangers of acidosis, a condition which precedes in every instance the onset of coma.* It has been interesting to determine the ratios used by various authorities. Joslin in his maintenance diets uses a lower ratio, on the side of safety, than many others, i.e., a ratio of approximately 1.0 to 1.0. Wilder uses a ratio of 1.65 to 1.0.Woodyatt

and many others use a ratio approximating 1.5 to 1.0, while Newburgh and Marsh have advocated a much higher ratio. *The higher the ratio of ketogenic elements in the diet, all factors considered, the greater will be the danger of acidosis.* On the other hand, the higher the ratio between the ketogenic and the antiketogenic constituents, the lower will be the amount of total glucose available in the diet, a fact of importance in considering the patient's individual tolerance for glucose as well as the tendency toward acidosis which some patients seem to manifest from apparently trivial causes.

For the past year and a half the diets used in the Los Angeles General Hospital have been based upon a ratio of 1.5 to 1.0, with results which have been satisfactory, due largely to the ingenuity of the tables arranged for the internes' reference by the medical director, Dr. P. Berman. The hospital treats about 150 diabetic patients yearly. The number of deaths from diabetes during the year July 1, 1923 to July 1, 1924 was 47, including all patients admitted in coma and those of advanced age, in many of whom the diabetic process was not the essential cause of death.

Children have a higher protein requirement than adults. They also require more calories. In the following tables the protein for children is figured at 2.0 gm. per kilo, the calories at 50 per kilo, and the ketogenic-antiketogenic ratio as 1.2 to 1.0. For adults the protein requirement is figured at 1.3+ gm. per kilo, the calories at 35 per kilo for maintenance, and the ketogenic-antiketogenic ratio as 1.27 to 1.0. The changes advocated, from the higher ratio, 1.5 to 1.0, to the lower ratio, 1.27 to 1.0, are believed to be in the direction of greater safety. The arrangement of diets is confusing for many physicians. It is believed that the matter is somewhat simplified by adhering to a plan such as the following tables give.

In beginning treatment of the usual patient with glycosuria and increased blood sugar, but who is not in acidosis or impending coma, rest in bed for a few days upon a 40–50 per cent maintenance diet usually suffices to render his urine sugar-free and to decrease his blood sugar. He is then advanced gradually

to the 60–100 per cent maintenance diets as rapidly as the severity of his diabetes will allow. If he cannot take a diet sufficient to maintain or increase his nutrition and remain sugar-free, insulin is used. *It should be realized that each patient requires individual adaptation of the diet to his tolerance to metabolize carbohydrates. In other words each patient is an individual problem. Success or failure as many times depends upon the supervision carried out in the home by those who are responsible for the preparation of the diet as upon the advice given by the physician.* This is especially true in the treatment of children. It is certain that more diabetic children are alive and in comparative health because of the intelligent care given them by their mothers in the matter of weighed diets than could possibly be the case if other well-meaning but less interested individuals were responsible for their care. *An essential part of the physician's duty therefore is the education of the mothers in the treatment of the disease.*

AVERAGE WEIGHTS OF CHILDREN[28]

Age	*Boys*		*Girls*	
	K.	*Lbs.*	*K.*	*Lbs.*
6	20.0	(44)	20.0	(44)
7	21.8	(48)	21.8	(48)
8	24.0	(53)	23.9	(53)
9	26.4	(58)	26.2	(58)
10	29.1	(64)	28.5	(63)
11	31.4	(69)	31.5	(69)
12	34.2	(75)	35.8	(79)

[28] Holt and Fales. *Am. J. Dis. Child.*, Chicago, 1921, xxi, 1.

Children

P. = 2.0 gm. per kilo.
Cal. = 50 per kilo.

Ketogenic-antiketogenic ratio = 1.2 to 1.0
G = total glucose.

		20 K. (44 lbs.)	25 K. (55 lbs.)	30 K. (66 lbs.)	35 K. (77 lbs.)
50 per cent Maintenance	P.	20	25	30	35
	C.	20	25	30	35
	F.	38	47	56	66
	G.	35	44	53	62
	Cal.	500	625	750	875
60 per cent Maintenance	P.	24	30	36	42
	C.	24	30	36	42
	F.	45	56	68	79
	G.	43	53	64	74
	Cal.	600	750	900	1050
70 per cent Maintenance	P.	28	35	42	49
	C.	28	35	42	49
	F.	53	66	79	92
	G.	49	62	74	87
	Cal.	700	875	1050	1225
80 per cent Maintenance	P.	32	40	48	56
	C.	32	40	48	56
	F.	60	75	90	106
	G.	57	71	85	99
	Cal.	800	1000	1200	1400
90 per cent Maintenance	P.	36	45	54	63
	C.	36	45	54	63
	F.	68	85	102	119
	G.	64	79	95	112
	Cal.	900	1125	1350	1575
100 per cent Maintenance	P.	40	50	60	70
	C.	40	50	60	70
	F.	75	94	113	132
	G.	71	88	106	124
	Cal.	1000	1250	1500	1750

Adults

P. = 1. 325 gm. per kilo.
Cal. = 35 per kilo

Ketogenic-antiketogenic ratio = 1.27 to 1.0
G = total glucose = Cal. × 0.067.

		40 K. (88 lbs.)	45 K. (99 lbs.)	50 K. (110 lbs.)	55 K. (121 lbs.)	60 K. (132 lbs.)	65 K. (143 lbs.)	70 K. (154 lbs.)	75 K. (165 lbs.)
40 per cent Maintenance	P.	21	24	26	29	32	35	37	40
	C.	21	24	26	29	32	35	37	40
	F.	42	48	52	58	64	70	74	80
	G.	37	43	46	52	56	61	66	71
	Cal.	560	630	700	770	840	910	980	1050
50 per cent Maintenance	P.	26	30	33	37	40	43	47	50
	C.	26	30	33	37	40	43	47	50
	F.	52	60	66	74	80	86	94	100
	G.	46	53	59	66	71	77	83	89
	Cal.	700	787	875	962	1050	1138	1225	1312
60 per cent Maintenance	P.	32	36	40	44	48	52	56	60
	C.	32	36	40	44	48	52	56	60
	F.	64	72	80	88	96	104	112	120
	G.	56	64	71	78	85	92	99	107
	Cal.	840	945	1050	1155	1260	1365	1470	1575
70 per cent Maintenance	P.	37	42	47	51	56	61	65	70
	C.	37	42	47	51	56	61	65	70
	F.	74	84	94	102	112	122	130	140
	G.	66	75	82	91	99	108	115	124
	Cal.	980	1102	1225	1347	1470	1600	1715	1837
80 per cent Maintenance	P.	42	48	53	58	64	70	75	80
	C.	42	48	53	58	64	70	75	80
	F.	84	96	106	116	128	140	150	160
	G.	75	85	94	104	114	124	133	143
	Cal.	1120	1260	1400	1540	1680	1820	1960	2100
90 per cent Maintenance	P.	48	54	60	66	72	78	84	90
	C.	48	54	60	66	72	78	84	90
	F.	96	108	120	132	144	156	168	180
	G.	85	96	107	117	127	138	148	160
	Cal.	1260	1417	1575	1750	1890	2047	2205	2362
100 per cent Maintenance	P.	53	60	66	73	80	86	93	100
	C.	53	60	66	73	80	86	93	100
	F.	106	120	132	146	160	172	186	200
	G.	94	107	117	130	142	153	165	178
	Cal.	1400	1575	1750	1925	2100	2275	2450	2625

Composition of Common Food Constituents[29]

		P.	C.	F.
Dried fruits (apricots, dates, figs, prunes)	50 gm.	2.0	35.0	1.0
5 per cent vegetables, fruits and nuts	100 gm.	1.0	3.0	0.0
10 per cent vegetables, fruits and nuts	100 gm.	1.0	6.0	0.0
15 per cent vegetables, fruits and nuts	100 gm.	1.0	9.0	0.0
20 per cent vegetables (potato, green corn)	100 gm.	2.0	20.0	0.0
Bacon, cooked, 4 crisp strips	30 gm.	5.0	0.0	15.0
Chicken, broiled	100 gm.	13.0	0.0	1.0
Meat, lean, cooked (round steak)	50 gm.	14.0	0.0	4.0
Meat, medium fat, cooked (roast, chop, ham)	50 gm.	11.0	0.0	12.0
Sweetbreads	50 gm.	9.0	0.0	6.0
Fish (halibut, mackerel, perch, trout) cooked	100 gm.	18.0	0.0	3.0
Fish, shell (lobster, crabs) cooked	100 gm.	14.0	1.0	2.0
Fish, canned (salmon, sardines)	100 gm.	23.0	0.0	17.0
Olive oil, lard, cottolene	15 gm.	0.0	0.0	15.0
Olives, green (6 med. size)	30 gm.	0.0	3.0	6.0
Shredded wheat biscuit (1)	30 gm.	3.0	22.6	0.4
Oatmeal (dry wt. 50 gms. = 350 gm. cooked)	50 gm.	9.0	33.0	4.0
Rice, boiled	100 gm.	3.0	24.0	0.0
Macaroni, cooked	100 gm.	3.0	16.0	2.0
Farina (dry wt. 50 gms.)	50 gm.	6.0	38.0	1.0
Bread (white, rye, whole wheat)	50 gm.	5.0	30.0	0.5
Bran muffins (special recipe[30]) each		3.0	3.0	6.0
Uneeda biscuit (2)	12 gm.	1.2	8.7	1.1
Cottage cheese	100 gm.	21.0	4.0	1.0
Cheese, American, Swiss, cream	100 gm.	26.0	1.0	33.0
Cream (18–20 per cent) 4 oz.	120 c.c.	4.5	4.2	24.0
Buttermilk, 8 oz.	240 c.c.	7.0	12.0	1.0
Buttermilk (made from whole milk) 8 oz.	240 c.c.	8.0	12.0	10.0
Milk, whole 8 oz.	240 c.c.	8.6	12.5	10.0
Milk, skimmed 8 oz.	240 c.c.	8.0	12.5	0.7
Butter (1 oz.)	30 gm.	0.0	0.0	25.0
Egg (1)		6.0	0.0	6.0
Peanut butter	20 gm.	4.0	3.0	12.0
Gelatin	10 gm.	9.0	0.0	0.0
Broth (beef, lamb, chicken)	60 c.c.	1.4	0.0	0.0

[29] The computations are figured from the publications of Joslin, from the Atwater and Bryant tables, and from Locke's "Food Values."

[30] Bran muffins: Unwashed bran 66 gm., soda ⅛ teaspoonful, salt ¼ teaspoonful, baking powder 1 teaspoonful, butter 25 gm., water ⅓ cup, eggs 2. Mix the dry ingredients, add the melted butter and water. Add egg yolks well beaten and fold in the stiffly beaten whites.

List of 5 Per Cent Vegetables,[31] Fruits and Nuts

Lettuce
Cucumbers
Spinach
Asparagus
Rhubarb
Endive
Marrow
Sorrel
Sauerkraut
Beet greens
Dandelion greens
Swiss chard
Celery
Mushrooms
Tomatoes
Brussels sprouts
Water cress
Sea kale
Okra
Cauliflower
Egg plant
Cabbage
Radishes
Leeks
String beans, canned
Broccoli
Ripe olives
Grape fruit
Avocado (fat 17 per cent)
Butternuts
Pignolias
Brazil nuts

List of 10 Per Cent Vegetables,[31] Fruits and Nuts

String Beans
Pumpkin
Turnip
Kohl-rabi
Squash
Beets
Carrots
Onions
Green peas, canned
Watermelon
Strawberries
Lemons
Cranberries
Peaches
Pineapple
Blackberries
Gooseberries
Oranges
Hickory nuts
Black walnuts

List of 15 Per Cent Vegetables,[31] Fruits and Nuts

Green peas
Artichokes
Parsnips
Lima beans, canned
Raspberries
Currants
Apricots
Pears
Apples
Huckleberries
Blueberries
Cherries
Filberts
English walnuts
Pecans
Almonds
Peanut butter

List of 20 Per Cent Vegetables[31] and Fruits

Potatoes
Shell beans
Baked beans
Green corn
Boiled rice
Plums
Bananas
Prunes

[31] Fresh or canned.

Clinical Comments on Diabetic Management

The discovery of insulin has made it no longer necessary to maintain diabetic patients in a state of undernutrition. In beginning treatment it has not been found wise to depend upon household or approximate measurements. It will be found much more satisfactory to use a food scale.[32]

In the treatment of patients with severe diabetes, especially those who manifest evidences of more or less disturbed kidney permeability, many difficulties may arise. In such patients it may be difficult to decrease the blood sugar to near the normal amount, although sugar may not be constantly present in the urine because of the higher renal threshold for sugar elimination. If the endeavor is made to restrict the carbohydrates for many such patients to a minimum, serious symptoms of acidosis may arise. The most important of such symptoms are: The presence of the so-called acetone odor to the breath, the presence of diacetic acid in the urine, the presence of hyperpnea or of nausea or vomiting, and an early tendency to sluggish speech or sleepiness. A high protein, high fat and low carbohydrate diet is a combination upon which many such patients do not do well. Patients with marked arteriosclerosis with or without hypertension will usually do better and be in safer condition if the protein is more or less restricted and the carbohydrates moderately increased, especially if the blood sugar is not high and sugar is present in the urine in only small amounts. For many patients of this type it has been found necessary in order to render them sugar-free to proceed very slowly in the restriction of carbohydrates. If the intake is very gradually restricted until the urine is sugar-free and then the tolerance is slowly built up it may be possible to keep the urine free from sugar for long periods without fasting. Should symptoms of acidosis arise, the fats should be restricted and the patient saturated with water. The attempt should be made to give one liter of water every four hours by mouth or by proctoclysis.

[32] A 500 gm. accurate food scale is recommended.

The injection of Ringer's solution into the vein is also a useful method. This should be given slowly.

It is most important that diabetic patients, when ill, even from trivial causes, should go to bed and take a glass of hot water, tea, clear coffee, broth, orange juice, or oatmeal water gruel every hour. The bowels should be emptied by an enema. The possibility of an impending coma should always be borne in mind.

Should coma develop, insulin, if available, should be used at the earliest possible moment. The dosage will depend upon the clinical condition of the patient, which includes, if time allows, the blood-sugar percentage. In general, 30 units may be given every hour for two or three doses, then, if necessary, every two or three hours for two or three doses more. At the Los Angeles General Hospital we use ordinarily 3 units per kilo of body weight in divided doses during the first twenty-four hours. The dosage is controlled by the blood sugar findings. *Smaller doses of insulin may be used if given by vein and are recommended as more dependable and the method of choice in coma.* In addition a 5 per cent glucose and 2 per cent soda bicarbonate solution may be given by the drip method per rectum, while glucose and orange juice or coffee may be given by mouth or by gavage. In order to prevent dehydration, fluids should be forced either by gavage or by hypodermoclysis of normal salt solution. In acidosis or coma, as Wilder has pointed out, the tissues are saturated with sugar. *There therefore is no advantage in giving more than moderate doses of sugar, usually in the form of orange juice.*

In general, for the uncomplicated diabetic patient, experience has indicated that it is much safer to begin treatment with the small dosage of one unit, increasing the dose daily as rapidly as possible. One unit of insulin will enable the patient to utilize approximately from 1.5 to 2.0 gm. additional carbohydrate. Joslin has reported one patient with 114 gm. carbohydrate tolerance whose blood sugar was lowered to the alarming point of 30 mg. by the injection of one unit of insulin. Allen has also reported a similar result. Such manifestations from

small doses of insulin must be rarely encountered but are of course possibilities. Woodyatt has reported that 90 per cent of his cases receive insulin but once daily before breakfast. The majority of clinicians prefer to administer insulin twice daily, twenty to thirty minutes before breakfast and supper. The doses of insulin required will depend upon the severity of the diabetic process, which includes the carbohydrate tolerance and the presence or absence of complications as well as the state of nutrition. There will be less loss of insulin if a tuberculin type of syringe with tightly fitting needle is used. There is probably an inevitable loss of 1 unit of U. 20 strength insulin per dose with the best syringe so far devised.

Should too large a dose of insulin be given with resulting rapid reduction in blood sugar, symptoms of nervousness, weakness, hunger, increased pulse rate and sweating may occur. These symptoms are relieved by giving sugar in some form, by the hypodermic injection of ten to fifteen minims of 1–1000 solution of adrenalin chloride, or, as Banting has recently reported, by the intravenous injection of calcium chloride. Banting has also reported that calcium lactate in 10-gr. doses may be given to children three times daily to prevent the development of shock during insulin treatment.

In case of contemplated operation upon a diabetic patient, nitrous oxide gas-oxygen should be the anesthetic of choice. As a general rule ether anesthesia is unsafe. It may be considered a safe rule before any operation upon a diabetic patient is performed, whether of major or minor degree, to restrict the intake of fats for a few days regardless of his apparent tolerance and freedom from symptoms of acidosis. Nearly every physician has seen serious sequelae follow minor operations, such as may have been performed by chiropodists, leading to gangrene because of failure to consider the possibility of an impending acidosis.

After operation upon a diabetic patient it usually is not wise, at least for the first few days, to give anything more than plenty of water by mouth or proctoclysis, or Ringer's solution by vein, albumin water, orange juice, fresh pineapple juice and

oatmeal gruel. The 5 per cent vegetables are not as a rule well tolerated during this trying period because of the stomach disturbance which is apt to occur.

References on Diabetic Management

Allen, F. M. *J. Metab. Research*, Morristown, 1922, ii, 866; *ibid.*, 1923, iii, 61. *Am. J. M. Sc.*, Phila., 1924, clxvii, 554; *Calif. & Western Med.*, San Francisco, 1925, xxiii, 1265.

Baehr, G., Lande, H., and Graves, L. G. *J. A. M. A.*, Chicago, 1924, lxxxii, 1509.

Christie, C. D. *J. A. M. A.*, Chicago, 1917, lxviii, 170.

Foster, N. B. *J. A. M. A.*, Chicago, 1924, lxxxiv, 719.

Harrop, G. A., Jr. Management of Diabetes, N. Y., 1924.

John, H. J. *J. A. M. A.*, Chicago, 1922, lxxviii, 103.

Jonas, L., and Musser, J. H., Jr. *Am. J. M. Sc.*, Phila., 1924, clxvii, 586.

Joslin, E. P. Treatment of Diabetes Mellitus. Phila., 1923. *J. A. M. A.*, Chicago, 1921, lxxvi, 79.

Kellogg, J. H. *J. A. M. A.*, Chicago, 1923, lxxxi, 823.

Newburgh, L. H., and Marsh, P. L. *Arch. Int. Med.*, Chicago, 1920, xxvi, 647; *ibid.*, 1921, xxvii, 699; *ibid.*, 1923, xxxi, 455.

Nicely, W. E., and Edmondson, C. C. *Am. J. M. Sc.*, Phila., 1924, clxvii, 570.

Nicholson, S. T., Jr. *Clifton Med. Bull.*, Clifton Springs, 1924, x, 101.

Petty, O. H. *Internat. Clin.*, Phila., 1923, ii, 37.

Sansum, W. D., Blatherwick, N. R., and Associates. *J. Metab. Research*, Morristown, 1923, iii, 641.

Shaffer, P. A. *J. Biol. Chem.*, 1921, xlvii, 433, 449; *ibid.*, 1922, l, 26. *Physiol. Rev.*, 1923, iii, 394.

Wilder, R. M. *J. A. M. A.*, Chicago, 1922, lxxviii, 1878.

Wilder, R. M., Boothby, W. M., and Associates. *J. Metab. Research*, Morristown, 1922, ii, 714.

Wilder, R. M., Boothby, W. M., and Beeler, C. *J. Biol. Chem.*, N. Y., 1922, li, 311.

Woodyatt, R. T. *Archiv. Int. Med.*, Chicago, 1921, xxviii, 125. *J. Metab. Research*, Morristown, 1922, ii, 793.

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