RENAL FUNCTION TESTS IN NEPHRITIS AND ALLIED CONDITIONS.

By E. C. DODDS, M.V.O., D.Sc., M.D., F.R.C.P.

(Courtauld Professor of Biochemistry in the University of London);

and J. DOUGLAS ROBERTSON, M.D., D.P.H.

(Clinical Chemical Pathologist to the Middlesex Hospital.)

[From the Courtauld Institute of Biochemistry, Middlesex Hospital.]

In health the kidneys are working well within the limits of their functional capacity in the same way as do the other organs of the body. The kidneys possess a very large reserve power and renal failure does not become apparent until the whole of this reserve power has been drawn upon. It is thus important to differentiate between renal impairment and renal failure. Let us assume that there are five million small units in each kidney which can function in a normal and healthy person, and let us assume that the smallest number of units at one time that can carry on the duties of the kidney is one million. If four and a half-million units are destroyed by disease, leaving only half a million units with a normal function, then renal failure will develop because the kidneys, with less units than their minimum requirements, cannot face their obligations. If on the other hand, two million units are destroyed by disease, then three million normal, healthy units continue to function. There is no renal failure because the functional threshold is exceeded, but the reserve power of the kidneys is diminished and renal impairment is present. Diagrammatically it can be represented thus:—

The ideal of all renal function tests is to put a severe load on the kidneys and so try out their reserve power, and by detection of renal impairment, take steps to rest the kidneys before the stage of renal failure has been reached.

TYPES OF RENAL TESTS.

These are of various types and include:—

1. Examination of the urine.
2. Examination of the blood.
3. Ability of the kidneys to concentrate and dilute.
4. Ability of the kidneys to eliminate foreign substances.
5. Simultaneous studies of blood and urine.

1. Examination of the Urine.

This includes measurement of the volume output in twenty-four hours, the specific gravity, and testing for protein and blood, and an examination of the centrifugalized deposit.

The **specific gravity** normally varies between 1015 and 1025, and depends upon the presence of dissolved urea, chlorides, sulphates, phosphates, and urates. To elevate the specific gravity by 0.001 at 15°C. the following amounts would be required to be added to one litre of urine.

Urea	...	...	...	...	3.595 grams.
Glucose	...	...	...	...	2.700 "
Sodium phosphate	...	...	...	...	3.792 "
Disodium phosphate	...	...	...	...	0.979 "
Sodium chloride	...	...	...	...	1.473 "
Sodium sulphate	...	...	...	...	1.405 "
Albumin	...	...	...	...	3.892 "

Thus so massive an albuminuria as 1% would raise the specific gravity by less than .003. There is also a correction for temperature—for every three degrees centigrade above 15°C. add .001 and conversely for every three degrees below 15°C. subtract .001.

The specific gravity may be low in certain types of nephritis.

Proteinuria. There are two main tests—(1) the boiling test and (2) the Salicylsulphonic Acid test. The boiling test is by far the easier to perform. It consists in boiling the top layer of a test tube full of urine after having made the urine clear by filtering and acid by the addition of one or two drops of 10% acetic acid. It is important to render the urine acid because on boiling, phosphates may appear owing to the lowering of the acidity by boiling off CO₂. The Salicylsulphonic Acid test is useful when only small quantities of urine are available. It consists in adding 2 to 3 drops of saturated salicylsulphonic acid solution to the urine. A precipitate forms in the presence of protein.

With regard to the test for proteins, it is as well to remember the presence of Bence-Jones proteose. This body can be detected by appearing at a temperature of 50°C. instead of round about 90° or 100° as in the case of protein. When boiling point is reached, Bence-Jones proteose dissolves. It re-appears on cooling.

The significance of proteinuria will be discussed later on in this paper.

Blood. Tests for Blood (chemical). To about one inch of urine is added about half an inch of glacial acetic acid. The mixture is boiled and cooled and one inch of ether is added to the test tube which is inverted several times. The ethereal layer is then allowed to separate, if necessary by the addition of water without shaking. This layer is pipetted into another test tube containing $\frac{1}{2}$ c.c. of alcoholic guaiacum solution and 2 c.c. of ozonic ether. In the presence of blood a blue solution will gradually develop. If the test for blood is carried out in this manner, the resulting blue colour is specific for blood. Such substances as oxidizing enzymes, iodides, salts of iron and copper being excluded by the above proceedings.

Deposit. The work of Addis (1925) has given an impetus to the quantitative study of urinary deposit. He has evolved a method which allows for an accurate record of definite types of casts. The enumeration of the casts is carried out in the following way.

Method. A normal breakfast on the morning previous to the test is allowed, but after that, the patient should be kept on a very dry diet, and all fluids are excluded. At 6 p.m. that evening, the patient empties his bladder, and this specimen is discarded. At 6 a.m. the following morning, the night's urine is collected—by catheter if the patient is a woman in order to ensure complete emptying of the bladder. The volume of the specimen is measured to within 2 c.c., the urine is then thoroughly mixed. Ten c.c. is then transferred to a specially graduated centrifuge tube. After centrifugalization of the tube for five minutes at 1,800 revolutions per minute, the supernatant urine is decanted. A drop is placed in a blood-counting chamber and the number of casts forming in twelve hours are as follows:—Casts—all hyaline—0—4,200; red cells—0—425,000; leucocytes—42,000—1,800,000.

In nephritis as many as 20,000,000 red cells and 30,000,000 leucocytes were found.

2. Examination of the Blood.

A study of the blood will demonstrate if the urinary constituents are accumulating through defective excretion by the kidneys. The group of substances most commonly determined is the non-protein nitrogenous constituents; this group includes, urea, uric acid, creatinine, creatine and amino-acid nitrogen. Other substances whose concentration is altered in impaired renal function are indican, chloride, phosphate, calcium, cholesterol and plasma proteins.

Urea. In renal failure the blood urea may rise above 500 mgms. per cent. as compared with a normal of 30 mgms. per cent. and the height of the blood urea will depend upon the degree of renal impairment provided of course the protein in the diet is controlled. There is no definite level at which uræmia appears but it is rarely present with a blood urea below 100 mgms. per cent.

Uric Acid. In severe renal impairment the uric acid may rise above 20 mgms. per cent. as compared with the normal of 2 to 3 mgms. per cent. It is believed by some investigators that the uric acid rises in the blood before the urea and is thus an earlier indication of impaired renal function, but this is not generally accepted.

Creatinine. Values as high as 20 or 30 mgms. per cent. may be met with in severe renal impairment. The normal range is 1 to 2 mgms. per cent.

Indican. Normally the blood contains 0.026 to 0.085 mgms. per cent. and in renal insufficiency it has been found to be raised above 7 mgms. per cent. Andrewes (1924) found the serum of uræmia patients gave a reaction with diazo reagent and it is probable that this reaction is due to the increased concentration of phenols in the blood stream.

Chloride. The normal plasma chloride is 550 mgms. per cent. of NaCl. It is frequently diminished under 400 mgms. per cent. in the azotæmic type of nephritis. In uræmia the low chloride content may be explained by the vomiting and consequent chloride loss.

Phosphate. The inorganic phosphate may rise from a normal of 3 mgms. per cent. to 10 mgms. per cent. or over.

Calcium. With marked nitrogen retention the serum calcium, normally about 10 mgms. per cent., falls below 8 mgms. per cent. This fall is believed to be due to phosphate retention. The serum calcium is also lowered when the plasma proteins are diminished.

Cholesterol. The normal blood cholesterol is 180 to 220 mgms. per cent. A figure as high as 600 to 800 mgms. per cent. may be found in the nephrotic type of nephritis. The reason for the raised cholesterol is not very clear but it is believed to be due to an attempt on the part of the body to improve the colloid osmotic pressure of the plasma rendered very low by the depleted plasma proteins. The hypercholesterolaemia disappears as nitrogen retention appears and the plasma proteins concentration rises.

Plasma Proteins. Normally these are found present in the following concentrations:—Albumin—5.5 grams per cent., globulin—2.0 grams per cent., fibrinogen—0.2 grams per cent. In chronic or sub-acute nephritis with oedema, the plasma proteins may be very reduced, the depletion taking place almost invariably in the albumin factor. The albumin may fall to under 1 gram per cent. The globulin seldom falls and is often raised above normal.

Very complete blood analysis can now be carried out on small quantities because of recent accurate analytical methods. It possesses many advantages over an analysis of urine in an investigation of renal function. Several fallacies must however, be remembered in interpreting the blood analyses, for its constituents may be raised in conditions other than renal impairment. Thus the blood urea may be raised in prolonged vomiting, intestinal obstruction, acute abdominal lesions and hæmorrhage. The uric acid may be raised in gout, the cholesterol raised in gall-stones, the plasma-proteins diminished in hæmorrhage, cirrhosis of the liver and malnutrition.

3. Ability of the Kidneys to Concentrate and Dilute Urine.

There are four types of tests.

1. The Urine Concentration Test. This depends on the fact that there is no serious impairment of kidney function if the specific gravity can be elevated to 1025 or over. At 6 p.m. the patient is given an ordinary supper but no fluids. At bed-time the bladder is emptied, urine discarded. On waking, he empties his bladder—Specimen No. 1. Remains in bed for 1 hour and empties bladder again—Specimen No. 2.

If renal function is normal, specific gravity is over 1030. Figures below 1020 show kidney defect.

2. The Urine Dilution Test. (Straus & Graunwald, 1917). The patient should remain in bed or quiet on a couch. The bladder is emptied. 1000 c.c. of water taken by mouth. At the end of four hours 1000 c.c. of urine should have been voided and the specific gravity of the largest volume passed should be in the region of 1002.

An excretion of less than 400 c.c. in the first four hours with a minimum S.G. of 1005 indicates impairment.

3. Mosenthal's Test-Meal (1915): Usual meals are taken at 8 a.m., 12 noon, and 5 p.m. with 600 c.c. of water at each meal. No food or drink between meals. Bladder emptied at 8 a.m. and discard specimen. Specimens are taken at 10 a.m., 12 noon, 2, 4, 6, and 10 p.m. and again at 8 a.m. Thus complete 24 hourly specimen obtained. The volume and specific gravity noted.

Notes:

Normal night urine should not exceed 400 c.c. with a specific gravity of 1016 or more. The total volume should exceed 1200 c.c. and the different specimens should show a maximum difference of at least 10 points. Increase of night urine to 700 c.c. or more is *abnormal*.

4. Urea Concentration Test. As little fluid as possible is taken from 8 p.m. the previous night. At 6 a.m. patient empties his bladder, and 15 grams of urea dissolved in 100 c.c. of water are taken by mouth. The bladder is emptied at 7 a.m., 8 a.m., and 9 a.m. The urea per cent. of all the specimens is estimated. It should exceed 2 per cent. In severe renal damage it may not reach 1 per cent. Test must be repeated if volume of any 1 hour exceeds 100 c.c.

Disadvantages.

1. It depends on absorption of urea.
2. It is possible to have a urea per cent. of over 2% when the blood urea is very high and when renal damage is severe.

4. Ability of the Kidneys to eliminate Substances.

This depends on the elimination in the urine of foreign substances injected into the blood stream. Such dyes as methylene blue, fuchsin, indigo carmine and phenolsulphonphthalein have been employed. The last named has been employed with success by Rowntree (1910). Bladder emptied. Injection of .006 gram of dye in 1 c.c. of water. 300 c.c. of water are drunk to ensure good flow of urine. Patient is catheterised. Urine allowed to drop into a beaker containing 2 drops of 25% NaOH. Red colour indicates first appearance of dye. The urine is collected for 2 separate hourly periods and amount estimated colorimetrically.

Normal: Dye present within 5 to 10 minutes.

1st hour—40% to 60%.

2nd hour—20% to 25%.

5. Simultaneous Studies of Blood and Urine.

(a) In 1912 Ambard and his co-workers were the pioneers in the study of simultaneous blood and urine determinations. They evolved the formula given below.

$$K = \frac{\text{Grams of urea per 1,000 c.c. of Blood.}}{\sqrt{\frac{\text{grams of urea per 24 hours excreted}}{24} \times \frac{70}{\text{Body weight}} \times \sqrt{\frac{\text{grams of urea per 100 c.c. of urine}}{25}}}$$

They found that K was .07 for normal people and readings such as 0.18 were found in nephritis.

(b) Addis (1925) suggested a much simpler formula:—

$$\frac{\text{mgms. of urea in 1 hour's urine}}{\text{mgms. of urea in 100 c.c. of blood}}$$

The normal ratio was about 50. This ratio fell as renal impairment developed.

(c) The most complete study was made by Van Slyke (1930) and his colleagues at the Rockefeller Institute in New York. The technique of the test is as follows. (Riches & Robertson, 1935).

The examination was performed in the morning after breakfast or in the afternoon after lunch. No preparation of the patient was carried out, except that no coffee was taken with the previous meal. The patient was at rest on a chair or couch during the test because exercise had been found to influence the results. Blood was withdrawn a few minutes before the end of the first hour. Patients were instructed not to void urine within three hours of their attendance, because of the uncertainty of emptying the bladder completely when containing only a small amount of urine. During the test the same difficulty of voiding urine was experienced at the end of the hour. This could be partially overcome by giving the patient a glass of water at the beginning and again at the end of the first hour. It was found that if a greater amount than this were drunk, while urination was made easier, there was a tendency to produce too low a urinary urea for accurate determination. It is most important that accurate time intervals be kept, and a stop-watch was employed to ensure accuracy to within a quarter of a minute. The duration of the period need not be one hour; it may be forty-five minutes, provided always the exact time is noted. It has been calculated that an error of one minute in the time interval produces an error of 1 per cent. in the final renal function estimate. Another source of error is incomplete emptying of the bladder. It has been our custom to catheterize all women and all prostate cases. An example is shown demonstrating the accuracy of the test when carried out as described above:—

H.J., aged 49. Diagnosis; Carcinoma of kidney.

10-10.02 a.m. — Catheterized and bladder emptied. Specimen discarded.

10.55 a.m. — Bled of 5 c.c. of blood.

11.02 a.m. — Bladder emptied by catheter (Specimen 1.).

12.02 a.m. — Bladder emptied by catheter (Specimen 2.).

Blood urea = 30 mgrm. per 100 c.c.

1st hour. — Urinary-urea = 0.4 grm. per cent. = 400 mgrm.

Urine volume = 160 c.c. per hour (2.66 c.c. per minute.)

$$\begin{aligned} \text{Maximum Clearance (Cm)} &= \frac{U}{B} \times V \\ &= \frac{400}{30} \times 2.66 = 35.5 \text{ c.c.} \\ \text{Normal is } &74 \text{ c.c.} \end{aligned}$$

$$\begin{aligned} \therefore \text{Percentage of normal} &= \frac{35.5}{74} \times 100 \\ &= 47 \text{ per cent. of normal.} \end{aligned}$$

2nd hour. — Urinary-urea = 0.6 grm. per cent. = 600 mgrm.
 Urine volume = 92 c.c. per hour (1.53 c.c. per minute.)

$$\begin{aligned}\text{Standard Clearance (Cs)} &= \frac{U}{B} \times \sqrt{V} \\ &= \frac{600}{30} \times \sqrt{1.53} \\ &= \frac{600}{30} \times 1.24 = 24.0 \text{ c.c.}\end{aligned}$$

Normal is 54 c.c.

$$\therefore \text{Percentage of normal} = \frac{24}{54} \times 100 = 44 \text{ per cent. of normal.}$$

Mean = 45.5 per cent.

The relative values and disadvantages of the various renal function tests employed may be briefly summarized.

1. Analysis on urine can be carried out with ease with no discomfort to the patient and with very little in the way of laboratory equipment. The presence of albuminuria however, does not indicate definitely an organic lesion. Benign forms of albuminuria such as orthostatic, lordotic, are associated with no functional impairment of the kidneys. Physiological albuminuria such as that following strenuous exercise, mental strain, or cold, are similarly benign in nature.

2. It has already been pointed out that nitrogen retention can be present in the blood in conditions other than renal failure.

3. Urine concentration and dilution tests, particularly the former, are held by some to be of the greatest value. It is, however, possible to have a highly concentrated urine when dehydration is present and there is sufficient head of urea in the blood—conditions met with in severely damaged kidneys. The urea concentration test depending upon absorption of urea by mouth also suffers from the objection of the former test. Further administration of urea by mouth is contraindicated in certain types of nephritis.

4. Perhaps the test of greatest value is the one described by Van Slyke and his co-workers (1930). This test, however, involves several analyses on blood and urine and facilities may not always be available for this. Where laboratory equipment is available it constitutes the most accurate index of renal function. Of the others, the simplest test of urine concentration is probably the next in value.

VALUE OF RENAL FUNCTION TESTS.

Renal function tests and in particular the urea clearance test has been employed in—

- (a) Diagnosing or excluding nephritis.
- (b) Following the progress of a case of nephritis.
- (c) Assessing the condition of patients with enlarged prostate.

Urea Clearance in Nephritis Based on the Observations of Van Slyke.

1. **Acute Hæmorrhagic Nephritis:** A fall to 50% or less is common. No prognosis can be obtained from the degree of functional impairment shown during the first two months. For instance I have seen as low as 10% recover. It is essential for a good prognosis that within four months after acute onset, the clearance, if it has fallen, shall have begun a consistent climb back towards a normal level. The functional recovery is not always complete. In all cases in which marked fall of blood urea clearance occurred during the initial months, and no definite tendency to rise followed within four months after onset, progress downwards to the active chronic or terminal stage followed. If renal function falls to 20% there can only be one outcome. When it falls to 5%—uræmia.

2. **Arteriosclerotic Type.** Low in all cases.

3. **Degenerative Type.** Normal with low tendency.

Enlarged Prostate.

Recently Riches and Robertson (1935) have employed the urea clearance in prostate surgery in preference to the customary blood urea examination. It is shown below how fallacious the blood urea can be.

Blood Urea.	Renal Function.	Notes.
32	35	Urine heavily infected.
32	70	After 5 months of suprapubic drainage and ketogenic diet.
30	70	After prostatectomy.

Widal (1904) has already demonstrated how misleading a blood urea analysis may be. By reducing the protein intake he obtained a normal figure for a blood urea in a patient ill with nephritis.

Blood Urea.	Protein Intake.
120	105 grams
193	105 grams + 20 grams urea
36	28 "
101	104 "

Also Mackay and Mackay (1927) have shown that blood urea does not begin to rise until the kidneys are reduced to half their functioning power.

REFERENCES.

- Addis, T., Jour. Amer. Med. Assocn., 1925, lxxxv, 163.
 Ambard, L. and Weill, A., Jour. Physiol. et Path. Gen., 1912, xiv, 753.
 Andrewes, C. H., Lancet, 1924, i, 590.
 Mackay, E. M. and Mackay, E. L., Jour. Clin. Invest., 1927, iv, 127.
 McLean, H. and De Wesselow, O.L.V., Brit. Jour. Exp. Path., 1920, i, 53.
 Mosenthal, H. O., Arch. Int. Med., 1915, xvi, 733.
 Riches, E. W. and Robertson, J. D., Brit. Jour. Surgery, 1935, xxiii.
 Rowntree, O. G. and Geraghty, J. T., Jour. Pharm. and Exp. Therap., 1910, i, 579.
 Straus and Graunwald, quoted in Barton's "Manual of Vital Function Testing," 1917, Badger, Boston.
 Van Slyke, D. D. et al., Jour. Clin. Invest., 1930, viii, 357.
 Widal, F. and Javal, A., Compt. Rend. Soc. de Biol., 1904, lvii, 301.