The Examination of Urine

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The examination of urine should un­
fallingly constitute part of the clinical examination. It should be looked upon in no other way than as part of a routine. The time spent by the clinician in carefully testing a patient’s urine is well re­
payed in so many instances. A diagnosis and a line of treatment may in quite a few cases have to be revised in view of the urine findings. It should always be born in mind that the urine does not reflect only what is happening in the kidneys, but in the whole body; suffice it to mention that the presence of glu­cose in the urine does not necessarily indi­cate a lesion in the kidneys.

The technical methods which will be outlined have been selected because of their practicability, simplicit y and selectivity. If carefully carried out they are as informative as the more elaborate tests.

Collection and Preservation of the Urine Sample

If any dependable data are desired regarding the quantita­tive composition of the urine, the examination of the mixed excretion for 24 hours is absolutely necessary, i.e. 8.00 a.m. urine is discarded then all urine passed up to and includ­ing that passed at 8.00 a.m. the next day is mixed, and a specimen is taken and tested.

When a 24-hour specimen is not prac­ticable, an early morning sample may be obtained. However, if one is testing for reducing substances and acetone bodies on a random sample, that passed about 1½-2 hours after a meal should be selected.

If possible all urines should be tested soon after they are passed i.e. within three hours; but if this is impossible such preservatives as a crystal of cam­phor of thymol to 8 ounces of urine, or better still 10 drops of formaldehyde, would not interfere with the tests for the major urinary constituents.

In some instances both the day and night urine may have to be tested se­parately for constituents e.g. Addison’s disease.

In several instances, especially in women, catheterization may be required to avoid contamination from vaginal or rectal discharges.

As in the physical examination of the patient, the examination of a specimen of urine is carried out in a methodical way following an established scheme. The scheme suggested is:

1. Physical Characters:— colour, turbidity; deposit; odour; volume; pH; specific gravity.
2. Chemical Analysis:—
   a) Tests for Protein
   b) Tests for Reducing Substances
   c) Tests for Ketone Bodies
   d) Tests for Bile pigments, Uro­bilinogen and Urobilin (when necessary)
   e) Test for Chlorides (when necessary)
3. Microscopical Examination.

The Normal Urine

The normal urine is subject to cer­tin variations, and variability in colour (within a certain limit), volume, pH and specific gravity, is an indication of nor­mality. The average colour is straw or light amber, the intensity depending on the degree of concentration. One to two litres of urine are excreted by the normal adult under average conditions, and volume depends naturally on such factors as fluid intake and water elimi­
nation through other channels. Odour is 'sue generis' unless alkaline fermentation has set in when it will be ammoniacal. The urine is clear; a faint turbidity or a light deposit may be due to a cloud of mucus. Under ordinary circumstances the reaction is slightly acid to litmus. The specific gravity range is 1.015-1.025.

Normal urine contains minimal amounts of protein chiefly mucoprotein, not detectable by ordinary tests. An ordinary specimen from a female may contain a detectable amount of protein often due to contamination from vaginal discharges.

Reducing substances may be present in traces in an odd specimen but this may be quite within normal limits. Ketone bodies are not usually present in detectable quantities.

Bilirubin should be absent, and urobilin and urobilinogen should not be present in any excess.

Microscopical examination should reveal on constituents whatsoever, or at most a very rare erythrocyte, leucocyte or hyaline cast. The presence of squamous epithelial cells alone is of no significance, and calcium oxalate or triple phosphate crystals may be present.

1. Physical Characters
   a) Colour: The straw colour of normal urine is due to the presence of a pigment urochrome, but in pathological states urines present a great variability in colour ranging from the extremely pale yellow specimen of chronic nephritis to the dark brown urine of haemoglobinuria. The presence of squamous epithelial cells alone is of no significance, and calcium oxalate or triple phosphate crystals may be present.

   b) Turbidity: Clear urine very rarely contains any pathological constituents. Even normal urine may show a bouyant cloud of mucus or a definite turbidity of phosphates on standing. Urines which show a definite turbidity when passed are always pathological.

   Turbidity may vary from a scanty uniform opalescence to the formation of thick floccules; this flocculation is usually associated with a deposit. Turbidity is due to substances in suspension, and such elements may be:
   i) bacteria ii) cellular elements (erythrocytes, leucocytes and epithelial cells iii) casts iv) amorphous and crystalline substances v) prostatic shreds, and vi) contaminants — notably yeast cells, cotton fibres, etc.

   When turbidity is marked, it is usually associated with a deposit, the quantity of the sediment being directly related to the density.

   c) A cloud of mucus may settle to the bottom of the container. Concentrated acid urines may show a bulky pinkish deposit of urates which dissolves immediately on warming to 60° C. A sandy deposit resembling red cayenne pepper is due to uric acid. A white flocculent deposit in alkaline or neutral urine is due to phosphates; this is cleared at once by the addition of a few drops of 5% acetic acid. Oxalates may deposit in both acid and alkaline urines and their true nature can only be detected on microscopy. Prostatic shreds can easily be identified on inspection as floating elongated threads.

   d) Odour: The faintly aromatic odour of a freshly voided urine specimen is due to undetermined volatile acids. On standing an ammoniacal smell is evolved as the result of fermentation. Peculiar odours may be detected following the ingestion of certain substances e.g. asparagus, turpentine, etc.

   e) Volume: Volumes above or below the normal range may be an indication
of serious disorder. There is an increase in the 24-hour volume in such conditions as diabetes mellitus, diabetes insipidus, nephrosis, and following the administration of drugs like digitalis, mercurial diuretics, 'Diamox', acetates and salicylates. Urine output is decreased in dehydration, vomiting, fevers, acute nephritis, heart failure and uraemia.

f) \( \text{pH or reaction to litmus:} \) The pH of a urine must be ascertained early in the examination, prior to the heat and acid test for protein and microscopy. The pH may vary within an appreciable range (Ph 4.8-8.0) in health depending on several factors. On an average the pH is 6.0 i.e. slightly on the acid side of neutrality; a value which is due to the presence of mono-sodium salts of phosphoric acid.

The urine may be alkaline following a heavy meal (alkaline tide) or after the ingestion of alkalies. In cystitis the urine is alkaline in reaction because of bacterial fermentation in the infected urinary bladder. In cardio-renal disease when the renal tubules fail in their compensatory mechanism, the reaction of the urine is well on the acid side.

For ordinary bed-side work litmus paper is used. Blue litmus paper turns red or remains blue according to whether the reaction is acid or alkaline respectively. Red litmus paper turns blue in alkaline urines. Sometimes both red and blue litmus paper change their colour, and the urine is then said to be amphoteric.

In routine laboratory practice use is made either of a series of liquid indicators or of special nitrazine indicator papers. With these methods it is the actual pH rather than the reaction that is determined.

g) \( \text{Specific Gravity:} \) The specific gravity varies directly as the amount of solids in solution and inversely as the volume. A large amount of sediment nearly always raises the specific gravity.

The normal range, under varying conditions of diet and fluid intake, and of fluid loss varies between 1.015-1.025. The specific gravity of a random sample is scarcely ever of any clinical significance.

In pathological states the specific gravity may vary from 1.001 to 1.060, and the relationship between it and volume may be lost. In chronic nephritis the specific gravity tends to be low and fixed from failure of the tubules to concentrate the excreted urine. A low specific gravity is also met with in diabetes insipidus. In acute glomerulonephritis, it is usually high because small volumes of a highly concentrated urine containing an excessive amount of protein, are passed. High figures of specific gravity are also met with in cases of diabetes mellitus when the sugar in the urine is raised.

The specific gravity is measured by means of the urinometer which is based exactly on the hydrometer; the scale being marked from 1.000 1.060.

Points to be observed in using the urinometer are:

i) The urinometer should float freely without touching the glass cylinder.

ii) The reading should be made from the bottom of the meniscus.

iii) No froth should be present.

iv) For small amounts of urine, dilute with an equal volume of distilled water and read the specific gravity after adequate mixing. Then multiply the last two figures of the reading by 2.

2. \( \text{Chemical Analysis} \)

a) \( \text{Protein} \)

The term "proteinuria" is preferred to the term "albuminuria" because albumin is not the only protein that may be present in urine. The commoner proteins to be found in pathological conditions are: plasma albumin, plasma globulin, mucus, fibrin, haemoglobin,
methaemoglobin, proteoses and Bence-Jones protein.

Tests for Protein

Several tests are described; the most convenient and satisfactory being the heat and acid test, and the salicylsulphonic acid test. As all tests depend upon the appearance of a turbidity or precipitate, it is essential that a clear specimen be obtained by filtering or centrifuging.

i) Heat and acid test: Urines should be slightly acid to litmus prior to boiling; an alkaline urine is to be acidified by the addition of a few drops of 5% acetic acid. If an alkaline urine is heated any protein present is changed into metaprotein—a soluble, and therefore, not coagulable substance. Once the medium has been acidified, metaprotein precipitates readily and is immediately coagulated by heat. The addition of too much acid should be avoided since it will cause the protein to go into solution.

For carrying out this test the tube should be three parts full. It should be held over a flame from the lower end, and at an angle, the upper layer being heated. Turbidity in the heated layer may be due to the presence of protein and/or phosphates; turbidity due to phosphates disappears after the addition of one drop of 5% acetic acid. If turbidity develops at a temperature of 50-60°C and disappears on boiling, Bence-Jones proteinuria should be suspected.

i) Salicylsulphonic acid test: Add 8 drops of 25% salicylsulphonic acid to 5 ml. of clear urine. Turbidity or precipitate indicates the presence of protein. More reagent may have to be added if the urine is alkaline.

This test has the disadvantage that it does not discriminate between Bence-Jones and other proteins, and may give a false positive reaction in the presence of uric acid and uroselectan (or iodoxyl). This can be differentiated in that a precipitate of uric acid redissolves on heating to about 60°C, and the turbidity formed with uroselectan is a white crystalline precipitate.

The results of these tests for protein are recorded as follows:

absent: no turbidity

slight trace: cloudiness against a black background

trace: cloud distinct without black background

† : cloud distinct and granular — no flocculation

‡ : cloud dense — marked flocculation

+++ : heavy precipitate to boiling solid.

b) Reducing Substances

The term 'reducing substances' is recommended in preference to 'sugars' because the chemical reagents used may be reduced by substances other than sugars. This occurrence is commonly met with in practice following the administration of salicylates, antipyrin, para-amino-salicylic acid, chloral, etc.

A number of tests have been devised for the detection of reducing substances in the urine, but only Benedict's and Fehling's methods are commonly used in clinical medicine. Benedict's qualitative test is preferred because it is the more sensitive. Both methods are based on the reduction of copper sulphate to cuprous oxide or hydroxide in the presence of a strong alkali. In both tests, a change in colour as well as the formation of a coloured precipitate constitute a positive reaction. The decomposition products arising from a large amount of protein may reduce cupric solutions giving rise to a false positive reaction. In such cases the proteins must first be coagulated by heating; the solution is then filtered, and the test for reducing substances carried out on the filtrate.

i) Benedict's test: The reagent con-
sists of copper sulphate, anhydrous sodium carbonate and sodium citrate in aqueous solution.

Five ml. of reagent are mixed with 0.5 ml. of urine (8 drops) and boiled in a water bath for five minutes (or over a flame for two minutes). Allow to cool (10-15 minutes). A faint green precipitate may be neglected, but a yellow or red precipitate indicates a definitely positive reaction.

A white or greyish-white precipitate is due to earthy phosphates and can be neglected.

ii) Fehling's test: The reagent consists of 1) copper sulphate in aqueous solution; 2) potassium sodium tartrate and potassium hydroxide in aqueous solution.

Mix about 1 ml. of solution 1) with an equal volume of solution 2), and in a separate test tube take about 2 ml. of urine. Heat both tubes over a flame, and when boiling pour the urine into the tube containing the reagent; allow to stand without further heating. A precipitate will soon develop if the test is positive.

Because of the stronger alkali present in Fehling's solution, a greenish precipitate is more likely to develop even in normal urines and therefore has to be neglected.

c) Ketone Bodies

Acetone, aceto-acetic acid, beta-hydroxybutyric acid and their salts are collectively known as ketone bodies. The presence of these substances in an abnormal quantity in the general circulation is referred to as 'ketonemia'; the term 'ketonuria' is applied when these substances are passed in the urine; the clinical syndrome being referred to as the state of 'ketosis'.

All urines containing reducing substances should be tested for ketone bodies. However, this does not imply that ketone bodies may not be present when reducing substances are absent. Ketonuria is usually associated with uncontrolled diabetes mellitus; it is also met with in conditions where there is restricted intake of carbohydrate or failure of its absorption. Thus ketonuria readily occurs in persistent vomiting from any cause, following short-circuiting operations on the bowel, persistent diarrhoea, etc.

Only qualitative tests for acetone and aceto-acetic acid are used as a routine in clinical practice. Aceto-acetic acid is the most toxic ketone body, and tests for its detection in a urine (Rothera's test, Gerhardt's test) form an important part of the chemical analysis.

i) Rothera's test (for acetone and aceto-acetic acid). Place about 1 ml. of crystals of ammonium sulphate in a test tube and add about 5 ml. of urine. Shake well until most of the ammonium sulphate has dissolved. Add 3-4 drops of a fresh 2.5% solution of sodium nitroprusside and 1 ml. of liquor ammon fortis. Mix well by shaking and allow to stand.

The appearance of a deep permanganate colour indicates a positive result, and its rate of development gives a rough indication of the amount of ketone bodies present. Rothera's test will detect both acetone and aceto-acetic acid; it is more sensitive for the latter. It detects one part of acetone in 10,000, and one part of aceto-acetic acid in 125,000 parts of urine. Because of this sensitiveness slight colour production should be ignored.

ii) Gerhardt's test (for aceto-acetic acid). To about 10 ml. of urine add solution of ferric chloride (10%) drop by drop mixing well after each addition. A white precipitate of ferric phosphate forms. Add more reagent slowly until the maximum precipitate has been obtained. Filter, and if the filtrate is free
from any marked reddish colour, add another drop of ferric chloride solution. If the phosphates have not been fully precipitated a further precipitate will result and the fluid must again be filtered, and more reagent is added till the maximum red colour is obtained.

A positive result is obtained if a characteristic Bordeaux-red colour results; orange or orange-brown shades should be disregarded. False negative results will be obtained if too much ferric chloride is added.

A positive Gerhardt reaction is given by aceto-acetic acid but not by acetone, and as this test is less sensitive than Rothera’s, a genuine positive result indicates a fairly marked degree of ketosis. On the other hand, Gerhardt’s test is not specific for aceto-acetic acid, and positive results are obtained by the excretion products of several common drugs as salicylates, aspirin, paraamino-salicylic acid, phenacetin, amido pyrin, etc.

A positive test due to one of the above-mentioned drugs can be differentiated in the following way. Boil the filtrate, obtained in Gerhardt’s test, for several minutes in a test-tube. On boiling, aceto-acetic acid is decomposed to acetone and carbon dioxide, and these in turn volatilized; the excretion products of drugs are unaffected. If the colour of the filtrate turns yellowish-brown, aceto-acetoc acid is present. Persistence of the original red colour indicates that the reaction is due to excretion products of one of these drugs.

d) Bile Pigments

Urine should be tested for bile pigments in the presence of jaundice or on account of the urine being coloured orange to deep-brown. On standing, urines containing bilirubin attain a froth easily forms on shaking. These features are only detectable if the urine is examined in daylight.

Normal urines contain no bilirubin or only a faint trace. Bilirubin is present in appreciable amounts in biliary obstruction, in hepatic disease, or when excessive haemolysis is taking place.

The tests for bilirubin to be described all depend on the oxidation of bilirubin to green (biliverdin) or blue (bilicyanin) pigments.

i) Iodine test: Take about 10 ml. of urine and pour slowly down the side of the tube 1 ml. of dilute iodine solution (1 part liquor iodii fortis diluted with 20 parts of water). A green ring developing at the line of junction of the two liquids indicates the presence of bile pigment.

ii) Rosenbach’s modification of Gmelin’s test. Filter 5 ml. of urine through a small filter paper; open out the filter paper and allow it to dry for some minutes. Pour a drop of concentrated nitric acid — a green ring indicates biliverdin, a blue ring bilicyanin.

iii) Fouchet’s test. Add 2 ml. of 10% barium chloride solution to about 10 ml. of urine; mix well and filter. Bile pigments are adsorbed by the precipitated barium sulphate and phosphate. The filter paper is allowed to dry and is then opened out on another filter paper, and two drops of Fouchet’s reagent (trichloracetic acid and ferric chloride in aqueous solution) are dropped in the centre. A green or greenish blue colour indicates the presence of bile pigment.

Fouchet’s test is the most reliable method for the detection of small quantities of bile pigments.

Gilles and Stokes (1945) have introduced a ‘methylene blue’ test: 5 drops of 0.2% solution of methylene blue are added to 5 ml. of urine. Normal urine appears blue on inspection by transmitted light; a urine containing bilirubin is coloured green. This test is quick but not specific for bilirubin. Furthermore, it is not as sensitive as the oxidation methods; and Fouchet’s test is recommended for the investigation of indi-
individual cases.

When excessive haemolysis is taking place or when the lives is diseased, urobilin or its chromogen, urobilinogen, appears in the urine. In practice urobilin is usually more abundantly present than urobilinogen because it is the oxidation product of the latter. Besides, there is no point in clinical medicine to distinguish between urobilin and urobilinogen as both have the same significance. In fact, in testing for the former the urobilinogen is converted into urobilin before proceeding further.

Normal urine contains minimal amounts of either, and when urine contains urobilin (but not urobilinogen which is colourless) it is coloured yellow or orange.

Ehrlich's reaction (for urobilinogen). To 10 ml. of urine add 1 ml. of Ehrlich's reagent (para-dimethyl-amino-benzaldehyde.) Shake well, and add 1 ml. of chloroform; mix and allow to stand. A cherry-red colour developing within 3 minutes in the chloroform layer indicates the presence of an excess of urobilinogen.

Schlesinger's test (for urobilin). To 10 ml. of urine add 2 drops of liquor iodii fortis; mix well (urobilinogen is oxidised to urobilin). In another test tube dissolve 1 gm. of powdered zinc acetate in 10 ml. of absolute alcohol; shake well. Pour the contents of one test tube into the other and mix well prior to filtration. A definite green fluorescence in the filtrate (viewed by transmitted light) indicates the presence of excess of urobilin.

A green fluorescence is also obtained when bilirubin is present. Urine containing bilirubin must therefore be treated with calcium chloride (add 1 ml. of 10% calcium chloride solution to 15 ml. urine), and filtered, before Schlesinger's test is carried out.

e) Chlorides

The detection (and estimation) of the level of chlorides in the urine is mostly required in cases of severe sweating, diarrhoea and vomiting, and in endocrine disturbances especially so in Addison's disease. It is also made use of to determine the point at which a deficiency has been made good.

The average amount of sodium chloride excreted is 12 gm. in 24 hours. The level of chloride per litre naturally depends on the degree of concentration of the urine.

The following is a simple test for sodium chloride which gives a semi-quantitative value:

Using a chloride-free pipette, take 10 drops of urine and one drop of 20% solution of potassium chromate. With a separate clean pipette add silver nitrate (2.9% solution) drop by drop, mixing well after each drop until there is a sudden change in colour from yellow to brown. The number of drops of silver nitrate required to reach this end point is equivalent to the number of grams of sodium chloride per litre of urine.

3. Microscopical examination

It has already been emphasised that microscopical examination of urine is an absolute necessity whenever protein is present. Further the nature of a deposit previously observed macroscopically, and the presence of turbidity have to be accounted for. No chemical tests are as satisfactory as a microscopical examination for the identification of crystals, cellular elements, the detection of blood and pus, etc.

A centrifuged deposit is usually examined unless the deposit is a very heavy one. A drop of the well-mixed deposit is placed on a slide and covered with a cover-slip. The preparation is then examined microscopically under the low and high power objectives. It is essential for easier and better identi-
ification that the condenser be lowered and the diaphragm be partly closed.

The elements which may be met with in a urinary sediment are listed in the accompanying table; only the more important of these will be discussed.

1. **Cells:**
   i) **Erythrocytes**
   ii) **Leucocytes** (or pus cells)
   iii) **Epithelial cells:** a) transitional, b) squamous.

2. **Casts:**
   i) **Granular:**
      a) fine, b) coarse
   ii) **Hyaline**
   iii) **Cellular:**
      a) **erythrocyte**, b) **leucocyte**, c) **epithelial**

3. **Crystals and amorphous chemical deposits:**
   i) **Uric acid** and urates
   ii) **Calcium oxalate**
   iii) **Phosphates**
   iv) **Carbonates**
   v) **Sulphonamide crystals**
   These are more rare: cystin, cholesterol, hippuric acid, tyrosine, leucine and xanthine.

4. **Miscellaneous:**
   i) **Mucus threads**
   ii) **Bacteria** and yeast cells
   iii) **Prostatic shreds**
   iv) **Parasites**
   v) **Foreign bodies**, like hairs, cotton fibres, starch granules, etc.

**Erythrocytes** appear as round discs with a more or less hyaline centre and often a halo can be made out around the periphery. The outline is usually well defined and regular, but may be crenated. Their size depends on the density of the urine. More than one cell per 10 high-power fields should be considered abnormal.

**Leucocytes** or **pus cells** are round discs with granular cytoplasm and a segmented nucleus; larger in diameter than an erythrocyte; often found in clumps or aggregates. The lobed nucleus may be made prominent by the addition of a drop of acetic acid. An occasional leucocyte may be found even in normal urines.

**Epithelial cells** may be derived from any level of the urinary tract as well as from the vagina. They are large cells with a prominent nucleus, a well-defined cell outline and a finely granulated cytoplasm. Transitional epithelial cells may have a polygonal, rounded or tailed outline, while (vaginal) squamous cells present an angulated cell border, are usually larger than transitional cells, and often occur in groups.

**Casts** develop within the renal tubules, and they therefore retain the shape and outline of this structure. Hence all casts show parallel, well-defined borders and rounded of fractured ends. Some confusion may arise when cylin-droids, hairs or collections of amorphous substances are present in a sediment. Cylindroids, however, possess a hyaline matrix and a tailed end; whilst hairs and collections of amorphous substances show only one of the structural characteristics of a cast.

Some casts owe their nomenclature to the cellular elements deposited on them, e.g. erythrocyte casts, leucocyte casts and epithelial casts. Granular casts are formed by the admixture of fragmented tubular cytoplasm and albuminoid material. Hyaline casts are refractile elements with a homogeneous albuminoid matrix. A few hyaline casts may be met with occasionally in a sediment of a normal urine. A number of hyaline casts, or the presence of other types of casts is an indication of renal damage, though not necessarily signifying nephritis.

**Crystals and amorphous chemical deposits.** Several types of salts may occur in urine either in a crystalline or in an amorphous form. The presence of these elements is only of significance if they are to be found in a freshly voided urine. More commonly these deposits are precipitated when the urine has stood for
some time. Certain substances are thrown out of solution in one reaction but not in another; others may crystallise both in an acid as well as in an alkaline reaction.

Deposits occurring in an acid urine:

Uric acid crystallises as barrels, plates, prisms or needles, and may form rosettes, and are often coloured in virtue of the adsorbed urinary pigments; they dissolve on heating at a temperature of 60°C, or on the addition of excess of alkali.

Urates may occur both in a crystalline and in an amorphous form. They dissolve on heating and on the addition of alkali. Sulphonamide crystals or their acetylated derivatives are more likely to crystallise out of solution in a concentrated acid urine. They usually crystallise as tufts, sheaths of needles depending upon the type of sulphonamide administered.

Deposits occurring in an alkaline urine.

Ammonium magnesium phosphate or triple phosphates crystals may be found when fermentation is present. Crystals of triple phosphates are seen under the microscope as ‘coffin lids’, ‘knife rests’, or as feathery crystals. They are dissolved on the addition of acetic acid.

The De Giorgio Prize

The University Authorities have been pleased to accept a prize of £25 given by Mr. Charles De Giorgio of Valletta to the student who

(a) qualifies in the first session in the examination of Therapeutics in the Final Course of Medicine and Surgery.

(b) And submits the best essay on a subject of Therapeutical interest approved by the Faculty Board of Medicine and Surgery.

The examination Board which will assess the merits of the essays, shall be appointed by the Faculty of Medicine and Surgery.

This Prize will be awarded for four consecutive final courses beginning from the final course 1955/58.

The Registrar of the University thanked Mr. Charles De Giorgio for his generous offer and we students who will be benefitting from this offer wish to express our gratitude and we are sure now that the examination of Therapeutics this year will be more interesting to us than it has been in the previous years; especially for those lucky students who will get this prize.