CLINICAL PATHOLOGY IN GENERAL PRACTICE

URINE TESTING IN THE SURGERY

BY

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This article is intended to describe as fully as possible, but without unnecessary elaboration, the procedure to be adopted in the examination of urine under the conditions normally present in the surgery. The tests chosen are those considered to be the most informative and selective. Practically all the tests are qualitative and they are often described as simple, but it is important to realize that this is no reason for doing it in a careless or haphazard manner. Experience will soon show that a test done with care gives a result which so far as the test goes is satisfactory; whereas a test made without such care often gives a result which leaves the practitioner in doubt, and he then embarks on a series of repeat or confirmatory tests, wasting time and destroying his confidence in the original test. This means that without necessarily employing precise measurements it is essential to keep as closely as possible to the prescribed quantities and conditions.

Necessary Apparatus and Reagents

Apparatus.—Tapered urine glass to hold about 6 fl. oz. (170 ml.); a microscope having $\frac{2}{3}$-in. and $\frac{1}{6}$-in. (1.7-cm. and 0.4-cm.) objectives; a supply of glass slides and coverslips; dropping pipettes with rubber teats; a small electric or hand centrifuge carrying 10-ml. tapered glass centrifuge tubes is a great asset but not absolutely necessary; urinometer reading from S.G. 1000 to 1060; a plentiful supply of clean test-tubes 6 by $\frac{5}{8}$ in. (15 by 1.5 cm.) and a test-tube rack to hold 12 tubes; Bunsen burner or spirit lamp; simple water-bath or saucepan; tripod and wire gauze; several small glass funnels 3 in. (7.5 cm.) diameter; filter paper No. 1 Whatman 12.5 cm. diameter; litmus paper; two 25-ml. graduated glass cylinders; two beakers of 50-ml. capacity.

Reagents.—Strong hydrochloric acid; strong nitric acid; strong ammonia (0.880); acetic acid (33%); strong solution of iodine (liq. iod. fort., *B.P.*); saturated solution of ammonium oxalate; 25% solution of salicylsulphonic acid; 10% solution of barium chloride; hydrogen peroxide (20 vol.); 10% solution of ferric chloride; 3% solution of silver nitrate; absolute alcohol or industrial methylated spirit; Benedict's qualitative reagent; Fouchet's reagent (this is made by dissolving 25 g. of trichloracetic acid in 100 ml. of water and adding 10 ml. of 10% ferric chloride solution); 5% alcoholic pyramidone; chloroform; zinc acetate in powder; sodium nitroprusside crystals; ammonium sulphate crystals.

Nature of the Specimen

It is important, particularly when testing for reducing substances, to know the time of the passing of the specimen in relation to the previous meal. The specific gravity of a urine will be influenced by marked variation in the fluid intake prior to the passing of the specimen. Traces of protein and increased cell content have a quite different significance in a fresh specimen passed in the surgery by a male patient from that of a specimen passed by a female patient, because the latter may have been contaminated by vaginal secretion. For most chemical examinations a fresh specimen is all that is necessary, but in the case of suspected haematuria, proteinuria, or infection it may be necessary to get a catheter specimen from a female patient.

Appearance and Colour.—Normal urine is of a straw colour which may be pale or dark merely because of variation in concentration. It is quite clear apart from an occasional cloud of mucus. Any turbidity is due to bacteria or suspended deposit which may be crystalline, amorphous, or cellular.

Reaction.—Normal urine is usually acid to litmus paper, but soon after a meal the alkaline tide may result in the production of an alkaline specimen which on standing will deposit a heavy cloud of earthy phosphates.

Specific Gravity.—In an average specimen this ranges from 1015 to 1020 and is influenced by the amount of fluid taken and by the loss of fluid by other channels than the urinary tract. The urine of a patient with chronic nephritis shows a more or less fixed low specific gravity, and limitation of fluid does not raise it appreciably. A specific gravity in the region of 1030 or over is usually due to the presence of sugar.

Deposit.—To the naked eye normal urine shows no appreciable deposit. Concentrated urines may show a deposit of urates, usually coloured pink, or a "Cayenne pepper" deposit of uric acid. The urates will dissolve immediately on warming the urine to about 60° C. Excess oxalates in a specimen of urine are usually recognized by microscopy; they appear in both acid and alkaline urine. Alkaline urines will usually have a phosphatic deposit readily soluble in a little acetic acid. Urates may also be deposited in a concentrated alkaline specimen.

Microscopical Examination of the Deposit

About 10 ml. of urine (which should be as fresh as possible) is centrifuged for 10 minutes at about 2,000 rev. per minute, and after removal of the supernatant fluid by decantation a drop of the fluid from the bottom of the tube, taken by a dropping pipette and teat, is transferred to a glass slide and covered with a coverslip. If a centrifuge is not available the urine should be allowed to stand for two to three hours in a tapered urine glass and a little of the deposit removed by a pipette and teat. Description of the cellular elements, casts, and crystals is usually of little help and it is best to learn to recognize them by practice on known specimens and comparison with the diagrams in textbooks of pathology. Apart from a marked excess of oxalates or uric acid the only crystals of importance are those of cystine; they are colourless, regular hexagonal plates, readily soluble in ammonia and hydrochloric acid but insoluble in acetic acid. Calcium oxalate crystals are envelope-shaped and are insoluble in acetic acid but soluble in hydrochloric acid. Uric acid crystals have always a red or brown colour and are insoluble in hydrochloric acid but soluble in solution of lithium or sodium carbonate. The commonest artifacts resembling cystine are maize-starch grains from dusting powders; these are readily recognized by their blue or black colour on the addition of solution of iodine.

Protein

Normal urine contains no proteins except a trace of mucin. Pathological proteinuria may be due to the presence of albumin, globulin, haemoglobin, methaemoglobin, or Bence Jones protein. As the tests depend on the appearance of a turbidity or precipitate, it is essential that the specimen should be filtered if it is not already clear. If the urine cannot be rendered completely clear, then some of the filtered specimen should always be used as a control to compare with the turbidity of the tested fraction.

Boiling Test.—For this test the urine should be acid to litmus; if not, it should be made so by the addition of just sufficient acetic acid. Boiling an alkaline urine containing protein may convert the latter into alkaline metaprotein; this does not coagulate and on the subsequent addition of acetic acid it becomes acid metaprotein, also in solution, and a false-negative result is obtained. Take about 10 ml. of urine in a test-tube and, holding the bottom with the first finger and thumb, boil the upper third. A cloud or precipitate may be due to protein or the appearance of earthy phosphates owing to the loss of carbon dioxide rendering the urine alkaline. Add two or three drops of 33% acetic acid. Phosphates will disappear, but the protein turbidity will remain and may become more intense. If on heating a cloud appears well below the boiling point and disappears partially or wholly on further heating and then reappears on cooling, Bence Jones protein is present. The boiling test is sensitive and reliable if the urine is clear and acid before the test is made.

Salicylsulphonic Acid Test.—To about 5 ml. of urine in a test-tube add 10 drops of 25% salicylsulphonic acid. The presence of protein is shown by a turbidity or precipitate. This is a useful test, as the original reaction of the urine does not matter. It has the disadvantage that it will not discriminate between Bence Jones protein and other proteins, and it is possible to get a false-positive reaction with urines containing iodoxyl after pyelography.

Blood

A chemical test for blood will indicate haemoglobin and its iron-containing derivatives such as methaemoglobin. To about 5 ml. of urine add three or four drops of 33% acetic acid and about one-fifth of its volume of solution of pyramidone in alcohol, pouring the latter down the slanted test-tube so that it forms a layer above the urine. Add four or five drops of hydrogen peroxide (20 vol.) so that it causes a disturbance at the junction of the liquids. A positive result is shown by the immediate development of a mauve colour at the junction of the liquids spreading up into the alcoholic layer. The colour soon fades unless the amount of blood present is excessive. If the colour appears very slowly and increases steadily for from five to ten minutes, it is probably a false reaction due to the presence of iodides. This can be confirmed by adding about 1 ml. of strong nitric acid to about 5 ml. of urine and then adding about 1 ml. of chloroform. Any liberated iodine will dissolve in the chloroform, giving it a mauve colour. The pyramidone test is quite sensitive but will not, of course, detect a few red blood cells such as can be seen in a microscopical examination of the deposit. It is a test for haemoglobin and will not distinguish haemoglobinuria from haematuria. Urines containing methaemoglobin will have a port wine or dark brown colour; its presence needs confirming by finding the characteristic absorption bands with a hand spectroscope. The use of this instrument for detection of pigments in urine is well described in Harrison's *Chemical Methods in Clinical Medicine.*

Reducing Substances

In the surgery the important points are, first, is a reducing substance present? and, secondly, is it glucose? If the latter, then further tests may be necessary to establish the diagnosis of diabetes. Take 5 ml. of Benedict's reagent, measured if possible in the graduated cylinder. Place it in a test-tube and add 0.5 ml. or eight drops of urine by dropping pipette (held vertically). Mix by shaking and boil thoroughly for at least one minute, or place in a boiling-water-bath for five minutes, and allow it to stand for a few minutes. A positive result is shown by the presence of a yellow or reddish yellow precipitate. A green colour or a greenish precipitate should be ignored, as it is probably due to a concentration of normal urinary constituents. It is of the greatest importance that the quantity of urine to that of Benedict solution should not exceed 1 to 10 or many false positives will result.

As glycosuria, whatever its cause, depends upon the blood level, when the urine is collecting in the bladder it is important to know that enough carbohydrate has been taken to cause such a blood level. The actual concentration in the urine will also be influenced by the amount of fluid taken. The best time to test for glycosuria is in the first specimen passed one and a half to two hours after a fairly liberal carbohydrate meal. If a negative result is obtained it is unlikely that the patient ever passes glucose. Other reducing substances are not dependent upon the previous carbohydrate intake, and reduction may be obtained in the presence of lactose, pentoses, laevulose, and derivatives of salicylates taken in the form of aspirin, sodium salicylate, or *para*-aminosalicylic acid. Pentoses are of no pathological significance, but their presence inevitably gives rise to a suspicion of diabetes. They may appear after their ingestion in fruits, but occasionally persistent pentosuria, unaffected by diet, occurs as an inborn error of metabolism.

A very rare cause of reduction is due to homogentisic acid in alcaptonuria. The reduction due to pentoses is not very pronounced and will be much the same in any specimens, fasting or otherwise. Glycuronates are very rarely a real cause of difficulty and will be related to the ingestion of large amounts of some drug such as morphine, chloral, etc. In adults, lactose, except in very small amounts, is found only in the urine of nursing mothers and is specially apt to occur at the time of stopping breast-feeding. Lactose may occur in the urines of infants with gastro-intestinal disturbance; very rarely galactose is found in the urines of infants made ill by the taking of milk in any form. Laevulose is uncommon but will sometimes be found in the urine of those consuming large amounts of honey.

The commonest false positive is that due to salicylates. Its incidence bears no relation to meals. If suspected it can be recognized by adding about 10 drops of solution of ferric chloride to about 5 ml. of urine and noting the intense purple colour.

Confirmation of the presence of glucose can be made by osazone and fermentation tests, and minute traces may be detected by chromatography; but the relation of the reducing substance to meals is usually sufficient evidence on which to base the diagnosis of glycosuria.

Acetone Bodies

The acetone bodies present in urine may be acetone, diacetic acid (aceto-acetic acid), and β-hydroxy butyric acid. Qualitative tests are available only for the acetone and diacetic acid. Ketonuria is usually associated with diabetes, but it can occur in any condition in which there is deprivation of or failure to use carbohydrate. Children rapidly develop acetone bodies in the urine when carbohydrate is cut off by vomiting or fasting.

Rothera's Test.—In a test-tube place ammonium sulphate to about the 1-in. (2.5-cm.) level and add about 10 ml. of urine. Shake well until most of the ammonium sulphate has dissolved. To this add three or four drops of a fresh solution of sodium nitroprusside, made by dissolving a few crystals in about 1 in. of water in a test-tube so that a fairly deep red solution results. Then add one drop of strong ammonia and mix well. A positive result is shown by the appearance of a deep purple colour. The test is given by both acetone and diacetic acid and is extremely sensitive. Because of this sensitiveness slight colour production should be ignored, and negative results indicate that there is no degree of ketosis. If the positive reaction is marked it is as well to perform a further test with ferric chloride, which, being relatively insensitive, has a greater significance if positive.

Ferric Chloride Test.—To about 10 ml. of urine add solution of ferric chloride drop by drop, mixing well after each addition, until the precipitated ferric phosphate appears to be maximum. Filter, and if the filtrate is free from any marked reddish colour add another drop of ferric chloride. If the phosphates have not been fully precipitated a further precipitate will result and the fluid must again be run

through the filter paper. To the clear filtrate add the ferric chloride solution drop by drop and notice if a red or reddish brown colour develops. It is very important to add the ferric chloride with care as excess destroys the colour of the iron salt formed with diacetic acid: false negatives are always due to excess of reagent. Of the acetone bodies only diacetic acid gives a colour. As the test is not very sensitive a genuine positive result indicates a fairly marked degree of ketosis. Drugs such as salicylate can give an intense purple or reddish purple colour but a negative nitroprusside test. If there is any doubt about the genuine nature of a ferric chloride test, take 10 ml. of urine in a cylinder and add 10 ml. of water, transfer to a small beaker, and boil until the volume is reduced to about 10 ml. Repeat the ferric chloride test. Any diacetic acid will have been converted to acetone and driven off by the boiling and no colour will now be obtained. Drugs will not be volatile and the result will be practically the same as in unboiled urine.

Bile Pigments

Bile pigments are present in urine only in jaundice, so a patient who shows no sign of pigmentation of the sclerotics is unlikely to have bile pigment (bilirubin) in his urine. Bile pigment may cause the urine to vary in colour from orange to deep brown; occasionally on standing there is a greenish tint due to oxidation to biliverdin. A characteristic of urine containing an appreciable amount of bile pigment is that, on shaking, the froth acquires a yellow tinge. Dark urines due to concentration, urobilin, blood pigment derivatives, and drugs do not show this. The yellow colour of the froth is not easily recognized in artificial light.

Iodine Test.—To about 10 ml. of urine add about one tenth of its volume of dilute iodine solution—that is, 1 part of liq. iod. fort. diluted with 20 parts of water. Pour the iodine slowly down the side of the slanted tube so that it forms a layer on the surface of the urine. A green ring developing at the junction of the two liquids is proof of the presence of bile pigment. This is a good but not very sensitive test.

Fouchet's Test.—For the detection of small quantities of bile pigment this test is the most reliable. To about 10 ml. of urine add about 2 ml. of 10% barium chloride solution. Mix well and filter. The precipitated barium sulphate and phosphate will absorb the bile pigments and after the filter paper has been allowed to drain it should be opened out flat on a white tile or another filter paper. Two drops of Fouchet's reagent are dropped in the centre. The presence of bile pigment is shown by the production of a green or greenish blue colour due to the oxidation of the bilirubin.

Bile Salts

The Sulphur Test.—Place about 50 ml. of clear urine in a beaker or urine glass and from a sprinkler or pepperpot sprinkle dry flowers of sulphur on to the surface. A positive result is shown by the sulphur particles streaming towards the bottom of the vessel. The beaker is best viewed by transmitted light; shaking must be avoided. This test cannot be done properly in a narrow vessel such as a test-tube. It is a sensitive test but can be given by other substances capable of lowering surface tension. Urine from a case of jaundice of some duration with much bile pigment will not necessarily show any bile salts as these are excreted only in the early days of the onset of obstructive jaundice and sometimes when a case of infective hepatitis is clearing up.

Urobilin

The presence of urobilin in the urine indicates either excessive haemolysis with excess production of the pigment or of some degree of liver damage. It is excreted partly as the colourless chromogen urobilinogen and partly as the yellow or orange pigment. On standing, urobilinogen is slowly converted to urobilin and the colour of the urine will deepen. Marked urobilinuria closely resembles the colour of bile pigment, but the froth is not coloured on shaking and gives no reaction with iodine or Fouchet's reagent. There is no point in trying to distinguish between urobilin and urobilinogen, and the best test is Schlesinger's, which gives a reaction with either.

Schlesinger's Test.—To about 10 ml. of urine in a test-tube add two drops of liq. iod. fort. or any comparable solution of iodine; mix well. In another tube place about 1 g. of powdered zinc acetate and add about 10 ml. of absolute alcohol or industrial methylated spirit. Shake well and mix the two fluids, pouring from one tube to another several times, and then filter. A positive result is shown by a filtrate that while yellow or pinkish yellow to transmitted light will show a definite green fluorescence by reflected light. The test is best carried out in daylight. This test is sensitive, and unless the fluorescence is readily perceptible an excess of urobilin can be excluded.

Chlorides

It is sometimes important to know if a patient is excreting a reasonable amount of chloride, and this can best be ascertained by adding to about 10 ml. of urine about 1 ml. of strong nitric acid and 2 ml. of solution of silver nitrate. Normal urine should give a heavy white curdy precipitate. No precipitate or only a slight turbidity or opalescence indicates a marked chloride deficiency in that specimen.

Next article on Clinical Pathology.—"Infected Urine," by Dr. Cuthbert E. Dukes.

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BUTAZOLIDIN SYMPOSIUM

CLINICAL AND EXPERIMENTAL OBSERVATIONS

A symposium on "butazolidin" (phenylbutazone) was held under the auspices of the Empire Rheumatism Council at the Royal Society of Medicine on November 13. The clinical section was under the chairmanship of Dr. W. S. C. Copeman, and Professor E. C. Dodds presided over the experimental section.

Dr. O. STEINBROCKER (New York) presented the results he and his colleagues had obtained with both short- and long-term butazolidin treatment. They found that it was a powerful analgesic, but that its antirheumatic effect, if any, was slight. They had compared the effects of intra-articular injections of butazolidin and hydrocortisone, and, although they had not observed any significant differences in the results, he was not prepared to attribute an antirheumatic action to phenylbutazone on this basis. The incidence of side-effects was 22% in the short-term therapy, and it was found that toxicity occurred in one-third of the cases on long-term treatment. At all periods administration of the drug was fraught with hazards. Dr. Steinbrocker emphasized that no serious toxic effects were encountered in the first 500 cases treated by his group, but that following this two major episodes occurred within one week. He warned against undue optimism and advised weekly analysis of urine and blood counts during the whole course of treatment.

Analgesic Action

Dr. J. P. CURRIE (Glasgow) compared the analgesic action of butazolidin with that of other analgesic antipyretics and newer analgesics. In conditions other than "rheumatism"