CLINICAL

BIOCHEMICAL METHODS

by

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PREFACE

THIS book describes the biochemical analyses in routine use in the laboratory of the Royal Berkshire Hospital. The facilities of this laboratory are similar to those available in most general hospitals. The procedures given are within the resources of such departments and the range of analyses dealt with, whilst not comprehensive, is typical of those in regular demand. The methods selected yield results in a reasonable time and with an accuracy sufficient for clinical requirements. Procedures which need expensive and highly specialized apparatus have been avoided. Alternative methods are given only where these are required for estimating the same constituent under different conditions.

The analyst using this book is assumed to have a working knowledge of analytical chemistry and the elementary procedures of clinical biochemistry. Thus no instructions are provided for standardizing acids, and familiarity with the use of general laboratory apparatus is assumed. With this proviso, accurate stepwise directions are given. All apparatus except standard equipment is specified and the manufacturer's name is given. A list of manufacturers' addresses is printed at the end of the book. Instructions for the use of special apparatus are to be found in the manufacturers' literature.

The book deals with experimental procedures, and no attempt at clinical interpretation is made. A Book List is provided: this gives some leading works of reference both in clinical biochemical theory and in laboratory methods. The reference at the head of each section is to the originator of the method, or to a later paper or review article in which the procedure has reached a more permanent form and which has served as the main source for the present modification. Where an author has published his own method in book form, reference is made to the book rather than the original paper.

How much a book of this type owes to the work of others hardly needs emphasizing; the References section and, more especially, the Book List are intended partly to serve to record my indebtedness. I wish to thank Dr J. Mills, chairman of the Reading Area Department of Pathology, for making this work possible. I should also like to thank Dr S. J. Baldwin, Miss V. A. L. Brews, Dr E. S. Halberstadt, Dr O. S.

Heavens and Mr E. W. Ingarfill for their generous help in the preparation of this book, and the publishers, Messrs Hilger & Watts Ltd, for their unfailing co-operation. I am grateful to authors and publishers of copyright material for permission to reproduce a number of tables and illustrations; individual acknowledgment of these is made in the text.

A. L. TÁRNOKY

May, 1957

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ACETONE BODIES IN URINE: ROTHERA'S TEST

QUALITATIVE

King and Wootton, 1956

Principle Acetone and acetoacetic acid form purple compounds with the alkaline decomposition products of sodium nitroprusside.

Specimen Fresh urine. If necessary it may be kept at 4°C overnight.

Method Fill ½ in. of a test tube with ammonium sulphate.
Add 1 ml of urine to give a saturated solution.
Add 4 drops of ammonia from a dropping bottle.
Add 4 drops of nitroprusside reagent from a dropping bottle.
Shake the tube and allow it to stand for about 5 minutes.

Results Report these as

Urine	Rothera	negative	..	if only a faint tinge of pink colour is visible;
Urine	Rothera	trace	..	if a weak pink colour is present; and
Urine	Rothera	+	..	where the test shows a strong purple.

Normal The test should be negative.

Reagents
1. Ammonium sulphate, laboratory reagent quality.
2. Ammonia (10% v/v) containing 10 ml of 0·880 ammonia, A.R., in 100 ml aqueous solution.
3. Nitroprusside reagent. Dilute 2 ml of conc. HNO_3, A.R., s.g. 1·42, to 200 ml with water. Add about 0·5 g of sodium nitroprusside, A.R., and dissolve by shaking.

Remarks
1. The test detects acetone in an approximately 1 : 10,000 dilution and acetoacetic acid in an approximately 1 : 100,000 dilution.
2. If the test is positive, Gerhardt's test for acetone bodies (p. 2) should be carried out.
3. Colours other than pink or purple should be ignored.

ACETONE BODIES IN URINE: GERHARDT'S TEST

QUALITATIVE

Hepler, 1949

Principle Acetoacetic acid gives a purplish coloured coordination compound with ferric ion.

Specimen Fresh urine. If necessary, it may be kept at 4°C overnight.

Method Fill ½ in. of a test tube with urine.

Add $FeCl_3$ solution from a dropping bottle until further addition causes no change in colour.

If a purple colour is formed, divide the solution between two test tubes. Heat one test tube at 100°C for about 2 minutes to drive off the acetoacetic acid.

Compare the two solutions.

Results If the solution gives no purplish or brown colour, report this as

Urine Gerhardt negative

If a purple colour is obtained which does not fade on heating, report this as

Urine Gerhardt negative

If a purple or brown colour appears which fades appreciably on heating, report this as

Urine Gerhardt positive

Normal The test should be negative.

Reagent Ferric chloride reagent, 3 g of $FeCl_3.6H_2O$, A.R., in 100 ml aqueous solution.

Remarks

1. The test is carried out if Rothera's test for acetone bodies is positive.
2. The test detects acetoacetic acid at an approximate dilution of 1 : 2000.
3. A number of drugs, mainly phenols, give a colour. These, however, are not volatile, and the colour does not disappear on heating.

ALKALI RESERVE OF PLASMA

Peters and Van Slyke, 1932

Principle The term refers to the reserve base available for holding acid, principally carbonic acid. The estimation is one of bicarbonate. This is first brought to the normal alveolar level of the plasma, is then displaced by lactic acid, and determined as carbon dioxide by measuring the gas pressure at a set volume.

Specimen Oxalated plasma separated from the cells within 5 minutes of taking the blood.

Method Place 2 ml of plasma in a 150 ml pear-shaped separating funnel. Connect the stem to the outlet end of a wash-bottle quarter-filled with glass beads and water (Fig. 1 below).

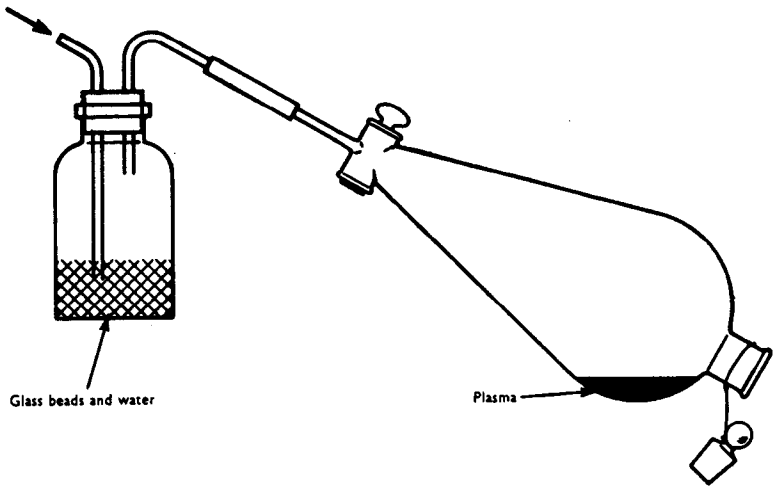

FIG. 1. Alkali reserve of plasma. *Saturation of plasma with expired air.*

Hold the funnel horizontally. Remove the stopper. Blow through the wash-bottle, taking a normal (not too deep) breath and continuing to breathe out as completely as possible. Replace the stopper while still breathing out, then close the stop-cock, and disconnect the funnel from the wash-bottle. Rotate the funnel to spread the plasma, and leave in a horizontal position ready for estimating.

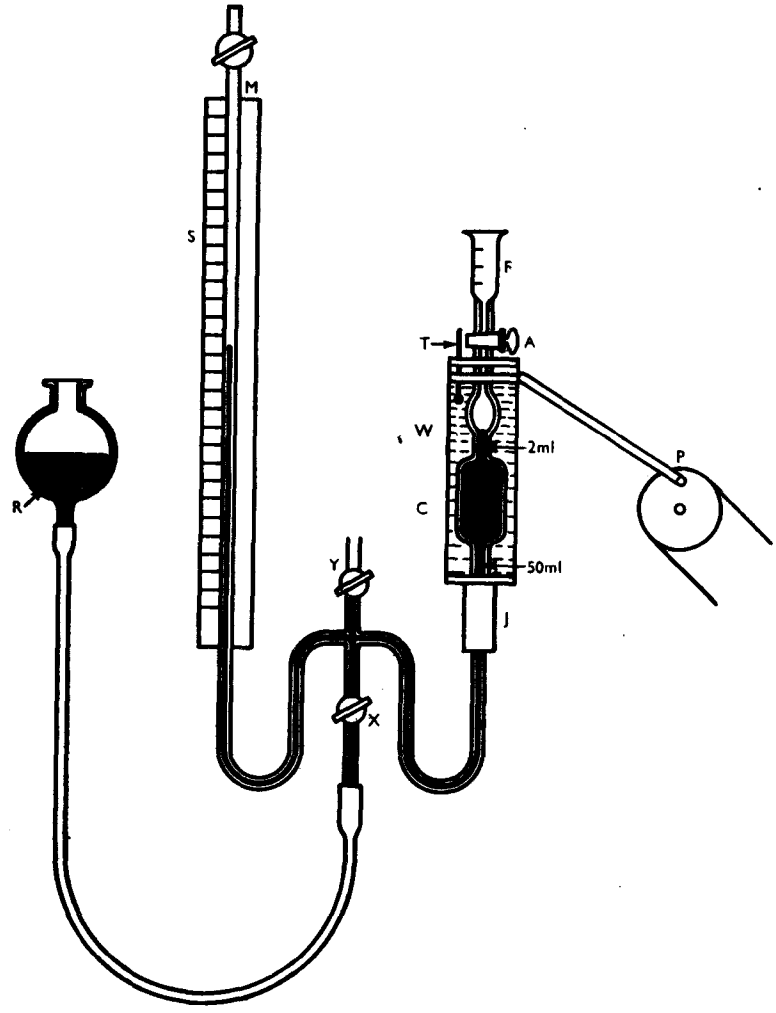

FIG. 2. Alkali reserve of plasma. *Manometric Van Slyke apparatus.* (From Bell, Davidson and Scarborough, 1953.)

Expel all air from the manometric Van Slyke apparatus (Fig. 2 opposite) and allow the mercury level to rise just above tap *A*. Close *A*. Add 2 drops of 2-octanol. Open *A* and lower reservoir *R* just enough to draw down most of the 2-octanol, leaving a short head in the capillary above *A* when it is again closed. Leave the reservoir in the lower position.

Pour approximately 1 ml of mercury into funnel *F*, and remove air bubbles from the neck of *F* by stirring with a nickel or steel wire.

Using an automatic pipette, add 1·5 ml of lactic acid to *F*.

Draw 1·0 ml of plasma into a long (not bulb-type) 1 ml pipette. Put a short closely fitting piece ($\frac{1}{2}$–1 cm) of rubber tubing over the tip of the pipette, allowing it to project a hair's breadth beyond the tip.

Stand the pipette in funnel *F*, pressing the tip on to the constriction above *A* to give an airtight fit. By means of tap *X*, draw the plasma slowly into the reaction chamber. Close *X* when the pipette is empty and a small air bubble appears below the tip. Remove the pipette with a sudden upward movement which also removes the air bubble. Open *X* and draw the contents of *F* into the chamber, leaving only a short head above *A*. Close *X* and *A*. Pour about 1 ml of mercury into *F*, and seal tap *A* by rapidly opening and closing it clockwise and then anticlockwise, drawing down most of the mercury.

Lower *R* further and empty the reaction chamber of mercury down to the 50 ml mark. Close tap *X*.

Shake the reaction chamber for 2 minutes $\pm$ 5 seconds; after switching off the motor make sure that the reaction chamber is in a vertical position.

Using tap *X*, raise the fluid level to the 2 ml mark. Record the reading on the manometer scale *S* to the nearest millimetre.

Raise the level of mercury in reservoir *R* slightly above the 2 ml mark on the chamber; equalize the pressures by opening and then closing tap *X*. Place *R* in the top position. Open tap *A*.

Gradually open *X* and allow the fluid level to rise slowly until it just fills the capillary above *A*. Close *X* and *A*.

Pour about 1 ml of mercury into funnel *F*, and remove air bubbles from the capillary by stirring with a wire. Replace reservoir *R* in the lower position. Open tap *X* halfway, and seal tap *A* by

rapidly opening and closing it clockwise and then anticlockwise, drawing down most of the mercury.

Lower *R* further, and draw the fluid level below the shoulder of the reaction chamber by means of tap *X*. Replace *R* in the top position.

Using tap *X*, raise the fluid level again to the 2 ml mark. Record the reading on the manometer scale to the nearest millimetre. Subtract this blank reading from the reading obtained in the test. This gives the combined pressure of CO_2 and air, in millimetres.

Record the temperature of the water-jacket to the nearest 0·5°C.

Results Obtain the alkali reserve level (mEq/l of plasma) corresponding to the temperature and pressure measurements by using the line chart (Fig. 3 opposite). Report this result as

Pl. alkali reserve ....mEq/l

to the nearest 0·5 mEq.

Normal 25·0–33·0 mEq/l.

Reagents

1. Octan-2-ol (*sec*-octyl alcohol, capryl alcohol), laboratory reagent quality.
2. Stock lactic acid solution, approx. 1 N. Dilute 42·5 ml of lactic acid, A.R., to 500 ml with distilled water which has been boiled in a hard glass vessel for 5 minutes.
3. Dilute lactic acid solution, approx. 0·1 N. Dilute 10 ml of reagent 2 to 100 ml with water treated as in 2.

Remarks

1. Mercury metal, A.R., is used in the apparatus. For method of cleaning used mercury see Harrison (1947).
2. The automatic pipette consists of a syringe with a screw-regulated plunger set at 1·5 ml, with a short pipette attached. This is incorporated in the stopper of the lactic acid bottle. The pipette is emptied by depressing the plunger, which is then raised by a spring into the filling position.

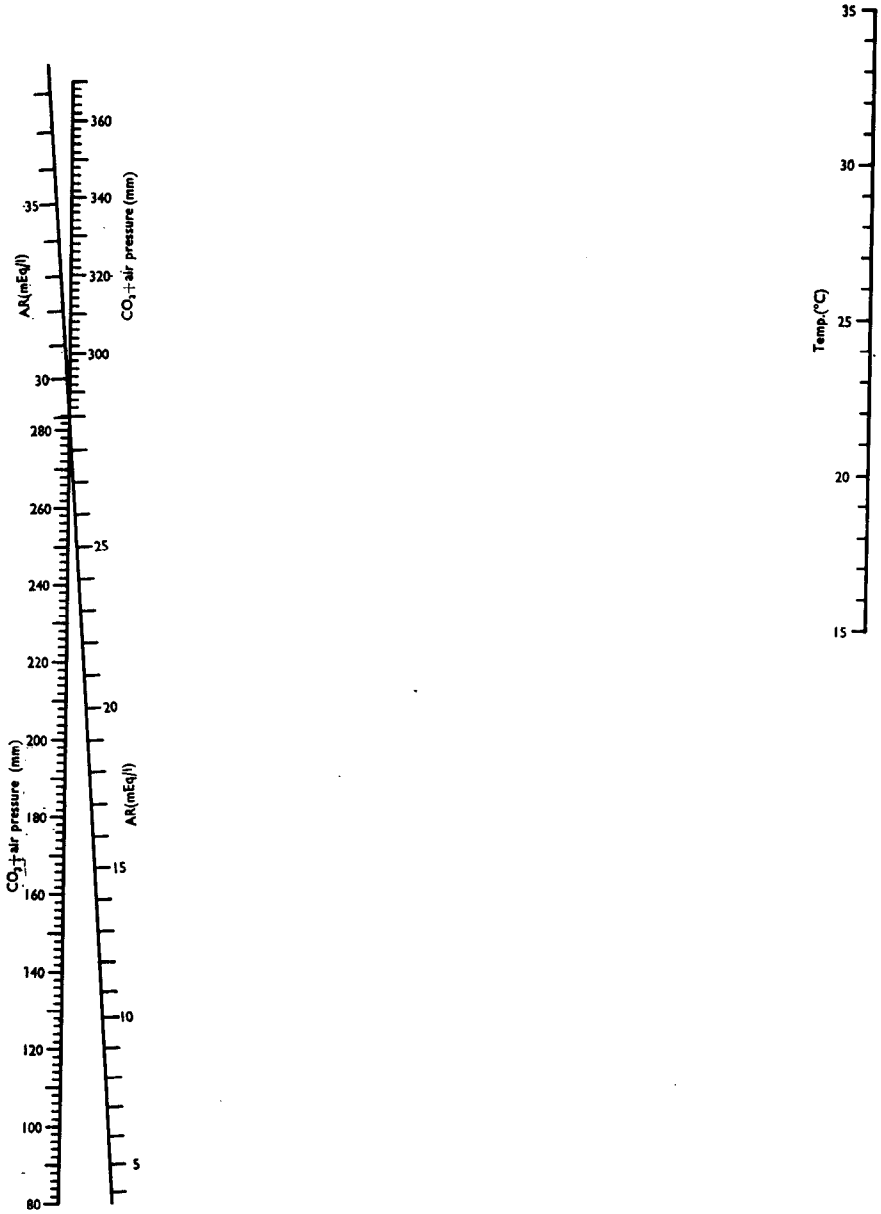

FIG. 3. Alkali reserve of plasma. *Line chart for calculating reserve level corresponding to the temperature and pressure measurements.* (From Peters and Van Slyke, 1932.)

ALKALI RESERVE OF PLASMA: EMERGENCY METHOD

Lehmann, 1951

Principle The term refers to the reserve base available for holding acid, principally carbonic acid in the form of bicarbonate. In the estimation the bicarbonate content of the plasma is first brought to its normal alveolar level. This is then displaced by sulphuric acid in a rough serial titration to pH 5·5.

Specimen Oxalated plasma separated from the cells within 5 minutes of taking the blood.

Method Place 1–1·5 ml of plasma in a 150 ml pear-shaped separating funnel. Connect the stem to the outlet end of a wash-bottle quarter-filled with glass beads and water (Fig. 1, p. 3).

Hold the funnel horizontally. Remove the stopper. Blow through the wash-bottle, taking a normal (not too deep) breath and continuing to breathe out as completely as possible. Replace the stopper while still breathing out, then close the stop-cock, and disconnect the funnel from the wash-bottle. Rotate the funnel to spread the plasma, and leave in a horizontal position ready for estimating.

Transfer a set of seven H_2SO_4 tubes to their corresponding place in the test-tube rack.

Add 0·20 ml of methyl red indicator to each tube.

Add 0·10 ml of plasma to each tube. Mix by thorough shaking. This gives a set of coloured solutions changing from yellow at the low-alkali-reserve end to red for tubes representing higher levels.

Results Select the orange solution which gives the best colour match with the pH 5·5 colour of methyl red. If the colour change along the series is not sudden enough to make the match-point obvious to the naked eye, fill a capillary pipette with the solution and use the B.D.H. Capillator card for comparison. Read off the alkali reserve value marked on the test-tube rack and report the result as

Pl. alkali reserve ....mEq/l (by emergency method)

to the nearest tube, or interpolate to an even number (2 or 8) between the two nearest tubes.

Normal 25–32 mEq/l.

Reagents

1. B.D.H. methyl red indicator ' for the B.D.H. Capillator.'
2. B.D.H. Capillator card: methyl red.
3. Sulphuric acid, 0·10 N. Add 1·6 ml of conc. H_2SO_4, A.R., s.g. 1·840, to 500 ml of water. Standardize, and readjust the normality.

10. Dilute 18·9 ml of reagent 3 to 100 ml with water.
15. Dilute 28·4 ml of reagent 3 to 100 ml with water.
20. Dilute 37·9 ml of reagent 3 to 100 ml with water.
25. Dilute 47·2 ml of reagent 3 to 100 ml with water.
30. Dilute 56·7 ml of reagent 3 to 100 ml with water.
35. Dilute 66·1 ml of reagent 3 to 100 ml with water.
40. Dilute 75·5 ml of reagent 3 to 100 ml with water.

Reagents 10–40 represent acid strengths equivalent to the alkali reserve levels to which they are numerically equal. Measure 0·10 ml of each into small tubes marked with these numbers. Stopper the tubes and keep these at room temperature.

Remarks

1. The tubes containing H_2SO_4 dilutions are stored in a box with compartments labelled 10–40. For the test, one tube of each dilution is transferred to a test-tube rack with a row of holes similarly marked.
2. The levels of alkali reserve corresponding to the different H_2SO_4 concentrations were derived from a series of parallel gasometric and emergency estimations.