CHEMICAL METHODS IN CLINICAL MEDICINE

THEIR APPLICATION AND INTERPRETATION WITH TECHNIQUES OF SIMPLE TESTS

By

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PREFACE TO THE FOURTH EDITION

No chapters have been added, but this edition contains much new work. Editing has again been drastic but a slight lengthening of the book has been unavoidable. It is impossible in a single volume of reasonable size to deal adequately with Literature in addition to Methods and Interpretations, even though description is limited to simple tests of clinical value. For that reason and because this is primarily a record of personal experiment, opinion or method of presentation, references have been restricted to special points or particular techniques and are mainly in the text.

Chapter I on apparatus has been extended most because it includes the new subjects of flame photometry, paper chromatography and paper electrophoresis. Furthermore, microscopy and polarisation microscopy have been amplified, and an overdue account of photoelectric colorimetry has been included. Elsewhere, recent work on bilirubin and conjugated bilirubin has been summarised, respiratory tracings in determinations of basal metabolic rates have been added and much of the chapter on Metabolism has been rewritten. Additions and alterations, however, will be found in most chapters and there are 40 fresh illustrations.

There are omissions which may cause disappointment, but some are deliberate. Thus estimation of urinary 17-ketosteroids is not described : hundreds have been made under the writer's direction but apart from a few low ones which are difficult to interpret, all have been normal. The empirical test of Robinson, Power and Kepler has been positive only when diagnosis of Addison's disease has been obvious from clinical findings supported by low values for serum sodium. A few more tests have been excluded as being technically unsatisfactory, or because the writer has nothing to add to accounts published elsewhere.

In this revision the author did the paging and is responsible for defects. He wishes he had done it in earlier editions because it made him detect several flaws in his manuscript, and appreciate some of the difficulties which publishers and printers have to surmount. He knows where tables and illustrations should be placed, but soon finds that division into pages may make use of an ideal position impossible. He quickly learns where figures or tables are out of proportion to length of text, and discovers that most of his sentences contain words which can be deleted, often with benefit. He alone may alter the text, and therefore has that great advantage when paging. On the other hand he is largely ignorant of much of the printer's art. Acknowledgement follows in the next section of the great help he had from others. It is hoped that the reader will conclude that a real

improvement has been made in spacing, in layout and in siting of illustrations and tables.

As before the author is greatly indebted to many persons for facilities, help, suggestions or valuable criticisms. Acknowledgements follow and apologies are offered to anyone whose name has been omitted.

G. A. H.

CHICHESTER, SUSSEX, 1957.

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G. A. H.

CHICHESTER, SUSSEX, 1957.

CONTENTS

CHAPTER I

SPECIAL APPARATUS

THESE are arranged alphabetically for convenience, and are put at the beginning to save repetition.

In every laboratory there should be a set of simple tools. Provision of more elaborate equipment, such as a lathe, is governed by demand and need for economy. A keen worker gradually collects his own tools, and this practice should be encouraged for many useful appliances can be made quite simply.

BENCH CLAMPS

A vertical clamp was introduced by the writer for assembly of cotton-mantle filters of transfusion sets (Fig. 1). It has several other applications, and is particularly useful when a vertical rod of a

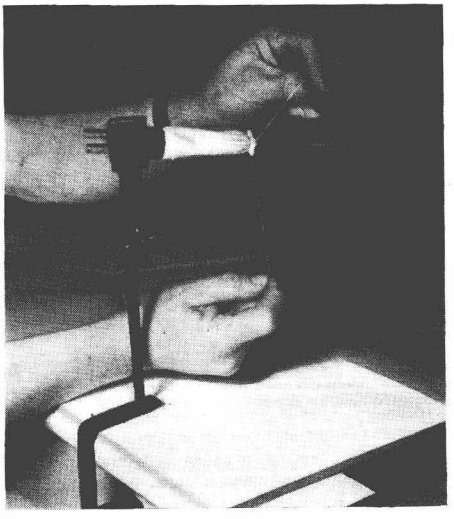

FIG. 1
Vertical clamp.
(*Photograph by W/Cmdr. R. Thorpe R.A.F.*)

retort-stand would get in the way ; being attached to the bench it cannot be knocked over. The free end of a clamp is threaded ; a bench clamp is drilled and tapped. The two parts are easily separated and occupy less storage space than the more usual retort-stand, boss-head and clamp.

Bench clamps can be used for several purposes ; thus if the vertical clamp of Fig. 1 is replaced by a suitably tapped rod, the bench clamp becomes a retort-stand. Addition of two 4 in. paper-clips, one grip of each being drilled and bolted to its opposite number to form a twinned clip, makes a burette-stand of convenient design (one end grips the rod, the other a burette).

An upper limb of a bench clamp can be lengthened by bolting to it a longer stout strip of steel, which in turn can be drilled and tapped to take two uprights holding a ring and gauze (a bipod instead of a tripod). To one, or both, legs a standard ring-clamp can be fixed for holding filter funnels. A third hole, mid-way between the two legs, may be drilled and tapped for a fixing screw of a bunsen burner after removal of its circular base-plate (a three-in-one apparatus is thus made, *viz.* filter-rack, bunsen and bipod ring).

It is not difficult to mount the urinary urea apparatus illustrated in Fig. 71, p. 131, on a bench clamp, and it is easy to think of other applications which are simple and cheap to make, and occupy little storage space or may be hung on nails out of the way.

BOTTLE MARKER

The apparatus illustrated in Fig. 2 was designed and made by the writer for scribing a ring on the outside of transfusion bottles ;

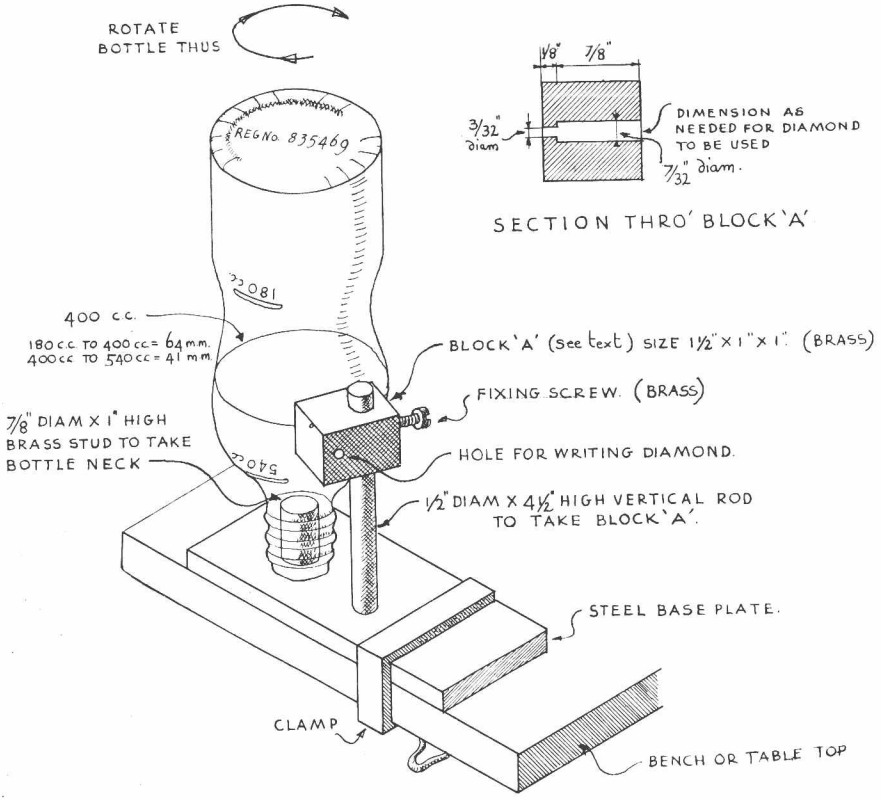

FIG. 2. Bottle marker. (*Drawn by Helen Low, A.R.I.B.A.*)

the mark indicated a capacity of 400 ml. of Seitz-filtered serum, which was the volume required for spin-freezing prior to drying.

A writing diamond was used, and never produced a crack in several thousand bottles. The drawing is self-explanatory. The apparatus is easily modified for marking bottles of other shapes and sizes.

BOTTLE SECTIONING

It is not easy to cut a bottle neatly in two to form a funnel from the upper or a dish from the lower part, but provided bottle (or jar) is of soft glass, it can be done by the following process.

Liquid paraffin is poured in to the appropriate level. The bottle is immersed in cold water, or in water containing ice, up to about the same level. A poker, or iron rod, is made thoroughly red hot and plunged quickly to the bottom of the bottle and held there for two or three minutes.

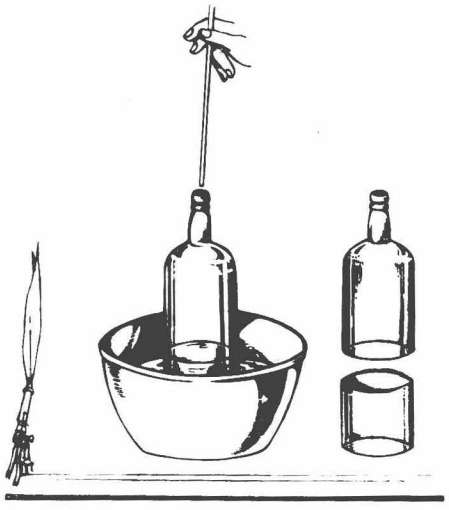

FIG. 3. Cutting a bottle in two.

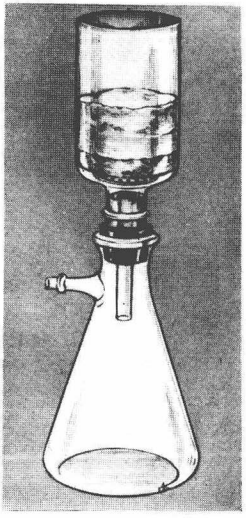

FIG. 4. Funnel made from a bottle.

The process (Fig. 3) may have to be repeated several times until suddenly there is a crack when the bottle is divided horizontally into two at the liquid level. Edges are razor-sharp and should be removed by emery paper. Rings may be cut in a similar way. Fig. 4 illustrates conversion of the upper part into a cylindrical funnel.

In a laboratory the chief difficulty is to make a poker really red hot. It must not be made white hot for fear of firing or exploding the liquid paraffin. The narrower the neck the more difficult it is to make a cut quickly. The writer has seen lime-juice bottles, milk bottles and jam-jars cut perfectly.

CENTRIFUGE, ELECTRIC

Description is unnecessary, but it is important to see that buckets with their contents are properly counterpoised, using a balance for the purpose (Fig. 5).

Current should be switched on, and resistance slowly taken out,

pausing at intervals to allow machine to gather speed. When stopping a machine, resistance should be put in first, and current switched off last, to prevent the next user inadvertently switching on current with resistance out of circuit, and thereby causing strain.

Periodically (*e.g.*, once a week or once a fortnight) a machine should be lubricated according to the maker's instructions. If no instructions are supplied, write to the makers.

The following substitute for the balance illustrated in Fig. 5 is simple to make, is cheap and saves space.

Four corners are cut off a rectangular piece of sheet-iron about 9 in. wide and 6 in. tall. The north arm is drilled to take a suspension-pin. The south arm is shaped to form the balance-pointer. The east and west arms are bent round to form holders for the centrifuge-buckets.

Centrifuge-casing is drilled and tapped to take a suspension-pin. Another piece of sheet-metal, shaped to form an upright arrow-head, is fixed to base of centrifuge-casing. Balance is suspended vertically above arrow-head : any adjustment required to make pointer and arrow-head line up exactly is made by movement of a small magnet from its initial location at centre of diamond-shaped balance.

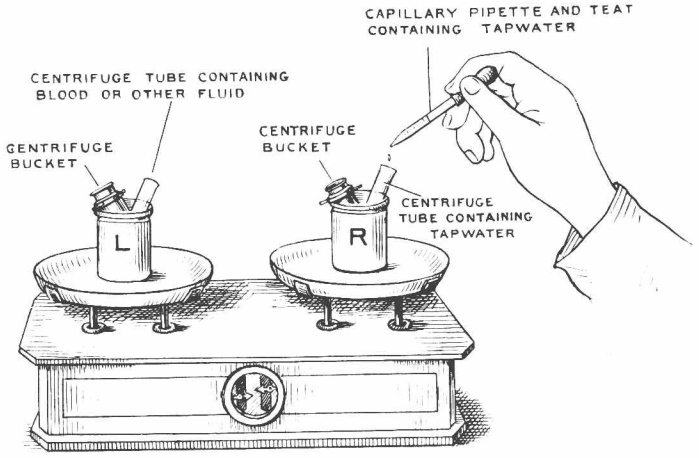

FIG. 5. Balancing centrifuge tubes.

COLORIMETERS

A colorimeter is an instrument for comparing colour-intensities of two solutions, one of which is termed the " Standard " and the other the " Unknown." The " Standard " contains a known amount of a given substance in suitable solution and is treated with a particular reagent to produce a coloured compound. The " Unknown " contains an unknown amount of the same substance in solution and is treated in exactly the same way as the " Standard."

There are two kinds, plunger and photoelectric. In the first, depths of two solutions are adjusted until the two fields appear equal in colour to the eye, *i.e.*, until the same amount of light is transmitted. In the second, depths are the same, and amount of light each transmits is measured by photoelectric current it produces in a selenium cell.

Both types come into action as the last step of an analysis, and obviously neither has any influence on errors made in earlier stages.

Colour of a solution varies with its depth and concentration of pigment in it.

$$\text{Colour} = \text{Depth} \times \text{Conc.}$$

For the Unknown, $\text{Colour}_u = \text{Depth}_u \times \text{Conc.}_u$ and for the Standard, $\text{Colour}_s = \text{Depth}_s \times \text{Conc.}_s$

In a *plunger colorimeter* Colour_u is made equal to Colour_s

$$\therefore \text{Conc.}_u \times \text{Depth}_u = \text{Depth}_s \times \text{Conc.}_s.$$

$$\therefore \text{Conc.}_u = \frac{\text{Depth}_s}{\text{Depth}_u} \times \text{Conc.}_s.$$

Since both depths are measured and concentration of Standard is known, concentration of Unknown can be calculated.

In a *photoelectric colorimeter*, depths of the two solutions are the same (usually 10 mm.), and colours are measured by photoelectric currents produced.

$$\text{Depth}_u = \text{Depth}_s.$$

$$\therefore \frac{\text{Colour}_u}{\text{Conc.}_u} = \frac{\text{Colour}_s}{\text{Conc.}_s} \text{ and } \text{Conc.}_u = \frac{\text{Colour}_u}{\text{Colour}_s} \times \text{Conc.}_s.$$

Since Conc._s is known and Colour_u and Colour_s are measured in terms of photoelectric current, Conc._u can be calculated.

Students are sometimes puzzled by the fact that with a plunger colorimeter

$$\text{Conc.}_u = \text{Conc.}_s \times \frac{\text{reading of Standard}}{\text{reading of Unknown}},$$

whereas with a photoelectric colorimeter

$$\text{Conc.}_u = \text{Conc.}_s \times \frac{\text{reading of Unknown}}{\text{reading of Standard}}.$$

Readings are of two different things, one of which (depth) is inversely, the other (colour or density—see below) directly, proportional to concentration.

A fuller explanation of colour is needed. When a solution of a pigment is put between a light source and the eye or photoelectric cell, pigment absorbs some and transmits the rest of the light to the retina or photo-cell (P.E. cell) respectively. It is transmitted light which acts on "receiver," it is absorption of light which is the property of a pigment. A colorimeter therefore may properly be called an absorptiometer. Degree of absorption is "density," or "optical density" of a pigment, and is analogous to density of a photographic negative.

This conception is important because optical density of a pigment is unalterable—it is a property of each molecule of pigment—whereas quality and/or quantity of light transmitted (*i.e.*, colour) is affected by alterations in the source. This is common knowledge, though not always appreciated fully; thus a particular coloured solution may have a different colour in daylight and artificial light

respectively, but the pigment solution and its optical density have not altered. Clearly, however, optical density and colour are related, because what is not absorbed is transmitted, or

percentage transmission = 100 — percentage absorption.

A coloured solution absorbs light of some wavelengths more than others ; therefore percentage absorption is higher when a source is selected which emits rays of the same part of the spectrum (alternatively, a coloured filter may be used in front of a white source). But the eye cannot see absorbed light ; what is seen is non-absorbed or transmitted light. Therefore a source is selected which is of a colour complementary to that which the eye sees. Thus blood looks red because it absorbs green and a green source of light is used. If a red source were employed percentage absorption by blood would be low. These principles are applicable to both types of colorimeter ; it is merely for convenience, and for simplicity of design, that white light illuminates a plunger colorimeter.

" White " light may be termed polychromatic, in that rays of all visible wavelengths are emitted. " Monochromatic " is used in several ways ; rays may be all of one wavelength, or of one colour and a few wavelengths (two from sodium), or of one colour and many wavelengths (*e.g.*, wide area of spectrum transmitted by a coloured glass filter).

PLUNGER COLORIMETER

Unless it has been shown by experiment that depth of colour is proportional to concentration over a wide range, Standard and Unknown must be approximately the same : they should not differ by more than 20 per cent. For this reason two or more Standards of different concentrations are frequently prepared simultaneously with the Unknown, and that which approximates most closely in colour is selected for final comparison. Beer's law seldom holds for polychromatic light, so the above rule is important if results are to be accurate.

Secondly, in developing colours in complicated mixtures such as blood or urine, it commonly happens that coloured compounds are obtained from substances other than the one under investigation ; though conditions are arranged to cause minimum of interference they do at times introduce different tints or modifications of the main colour, so that comparison is inaccurate or impossible. Thirdly, both Standard and Unknown should be absolutely clear ; occasionally a precipitate is formed which may make colorimetric comparison impossible or inaccurate. Difficulties due to variation in tint, or to opacities, are particularly likely to be encountered in pathological work. The Standard is usually a treated solution of the pure substance to be estimated, in water or some other solvent (volatile solvents are obviously undesirable, but sometimes have to be used). The Unknown is a treated solution of the same substance in some complicated solvent such as urine, urine filtrates, protein-free

filtrates of blood, and so on. Theoretically the Standard should be prepared by dissolving x in the Unknown deprived of its content of x, but this is generally impossible.

"Artificial Standards" are sometimes used. These are of two main types: (i) a solution of another chemical which has the same colour as the solution of the substance to be estimated (*cf.* Standard in van den Bergh's test, Chapter XII); (ii) glass discs of appropriate colours. The above points are important in indicating limitations of colorimetry. In spite of them, however, colorimetric methods are valuable, and particularly in chemical pathology, on account of their relative simplicity and the speed with which repeated estimations can be performed. Moreover, colorimetric methods are often applicable to much smaller quantities of fluid (*e.g.*, blood) than the more orthodox volumetric or gravimetric methods. In medicine it may be a question of a colorimetric method or none at all.

The following are essential **practical instructions** (Klett type of apparatus):—

(i) Clean and dry both cups and plungers.

(ii) Adjust (if necessary) zero settings. Rack up each cup till it touches bottom of plunger, and see that O corresponds to O on each side. If not, reset scale. In the Klett type make sure that base of cup sits properly on base of holder.

(iii) Fill both cups with Standard. Set each at, say, 20 mm., and adjust mirrors till illumination is even.

In the newer types there is an electric bulb in the base as direct light source, which is adjusted till illumination of the half-fields is equal. A set-screw is provided which is loosened initially, and screwed home when adjustment has been made.

(iv) Leave Standard in right-hand cup. Tip back or throw away solution from left-hand cup, draining it as much as possible. It is not necessary to dry it, as one drop diluted by several millilitres of Unknown introduces no appreciable error. Fill left-hand cup with Unknown and set at 20 mm. Rack up or down right-hand cup containing Standard until it matches. Calculation is simplified by this setting of the Unknown at a fixed level (*cf.* calculation of blood-sugar (Folin and Wu) in Chapter VII).

Some workers take a single reading, but most prefer the average of five or six. Prolonged staring must be avoided lest retinal fatigue occurs. The first reading is commonly slightly different from the rest, and in that case may be discarded. There are often slight variations in readings obtained by different individuals; thus one assistant almost always obtained a slightly higher average than the writer, though both had had ample practice. The personal factor, and retinal fatigue, have been regarded as objections to colorimetry, but actually variations expressed as a percentage of the final result are small, usually less than 2 per cent. Volumetric titrations may differ by only 1 drop, colorimetric readings by 2 or 3 mm., but for comparison they must be expressed in common terms.