Microdetermination of calcium and magnesium in biological materials

C. H. Bowden¹ and Valerie J. Patston
From the Department of Chemical Pathology, Westminster Medical School, London

SYNOPSIS The use of the dye calcon (1-(2-hydroxy-1-naphthylazo)-2-naphthol-4 sulphonic acid) for the estimation of calcium using E.D.T.A. and a commercial photoelectric titrimeter is described. The interfering effects of magnesium and phosphate have been overcome. The method has been extended to estimations on biological materials.

Results on 55 sera show that the E.D.T.A./calcon method gave slightly lower results (–0.15 mg./100 ml. ± 0.029) than the oxalate precipitation method.

Magnesium may also be estimated by incorporating the use of Eriochrome black T.

The dye calcon (1-(2-hydroxy-1-naphthylazo)-2-naphthol-4 sulphonic acid), which was investigated by Hildebrand and Reilley (1957) and adapted for use in serum calcium estimations by Golby, Hildebrand, and Reilley (1957), appears to be a real improvement on murexide, in both stability and colour change. Aqueous solutions of calcon produce a blue colour between pH values of 7.4 and 13.5. Below pH 7.4 and above pH 13.5 the indicator is red. At approximately pH 13, where magnesium is quantitatively precipitated as the hydroxide, only calcium ions react with calcon to form a red coloured complex. Thus under these conditions the calcium-calcon complex can be titrated with E.D.T.A. (ethylene diamine-tetra acetic acid) from a red to a pure blue end-point without interference from magnesium.

In our experience, however, the visual end-point described by Golby et al. (1957) is not sufficiently sharp to give highly accurate results. Also as sera vary greatly in tint the colour of the end-point will vary accordingly. We therefore made use of a commercial titrator and galvanometer, which gave a far more accurate determination of the end-point. A single calcium estimation using this apparatus takes less than three minutes. The method has been adapted satisfactorily for calcium estimations in serum, urine, food, and faeces.

EXPERIMENTAL

APPARATUS The EEL titrator (Evans Electroseelenium) used in conjunction with an EEL Unigalvo type 20 galvanometer was used throughout the work described. The instrument consists basically of a colorimeter with a built-in magnetic stirrer. Flanged beakers (4 ml. capacity) are supplied which are correctly located in the light beam which is focused at the centre of the solution in the beaker. Various Ilford filters can be selected for use as necessary.

As no error in the end-point is caused by moderate dilution of the sample it was unnecessary to use a micrometer syringe when titrating with E.D.T.A. Therefore a 2 ml. Excello automatic micro burette® graduated in 0.01 ml. divisions was used. The burette was modified by fitting it with a diaphragm plastic tap® which permitted accurate 0.01 ml. additions. The burette jet was either drawn out finely or the tap fitted with a hypodermic needle.

OPTICAL CONDITIONS FOR TITRATION The exact position of the absorption peaks, the molar extinction, and the stability of the colour of the free calcon and of the calcium complex are all dependent on pH. Above pH 13 the stability and the extinction of the free calcon is greatly reduced (Hildebrand and Reilley, 1957). However, the advantage of avoiding both the necessity of neutralizing acid extracts before titration and magnesium interference by using high pH levels outweighs the disadvantages of decreased dye stability and reduced optical density values. Under the working conditions described below the free dye had a loss of optical density of 1% at five minutes at wavelength 490 rising to 20% at 20 minutes. Under the same conditions the calcium-calcon complex showed no significant change.

DETERMINATION OF THE END-POINT FROM TITRATION CURVES Figure 1 shows the titration curve of a standard

¹Present address: Royal Air Force Institute of Pathology and Tropical Medicine, Halton, Aylesbury, Bucks.
Received for publication 29 September 1962.

²W. G. Flaig & Sons Ltd., 39, Waterloo Road, London, N.W.2.
³Townson and Mercer, Croydon, Surrey.
Microdetermination of calcium and magnesium in biological materials

caution solution using an Ilford 603 filter and galvanometer transmission scale.

The end-point can be determined without drawing a titration curve by noting the greatest difference in transmission scale deflection for each 0-01 ml. of E.D.T.A. added. This corresponds to the maximum rate of deflection and the end-point is taken as the second of the two readings showing a maximum difference.

REAGENTS The following were required:—

Stock calcium solution (0-01251M or 50 mg. Ca/100 ml.) 1-251 g., CaCO₃ anhydrous AR (dried at 120°C. and stored in a vacuum desiccator) is added to 150 ml. distilled water. After the addition of 25 ml. NHCl the solution is made up to 1 litre.

Dilute calcium working solution (5 mg. Ca/100 ml.) Dilute the stock calcium solution 1:10 with distilled water.

Diamino-ethane-tetra acetic acid solution (E.D.T.A.) (0-0025 M) Diamino-ethane-tetra acetic acid disodium salt, 0-9306 g., is dissolved in 1 litre of distilled water.

Stock calcon indicator Calcon,⁴ 50 mg., is dissolved in 10 ml. methanol AR.

Dilute calcon indicator Dilute stock solution 1:25 with methanol.

2N Sodium hydroxide All aqueous reagents are prepared using ion exchange water and stored in polythene bottles.


STANDARDIZATION OF E.D.T.A. SOLUTION Into the 4 ml. beaker pipette 1 ml. dilute calcium standard, and add 0-5 ml. 2N NaOH and 0-2 ml. dilute calcon indicator.

With medium to maximum sensitivity setting and using the Ilford 603 filter, titrate until the end-point begins to change colour, and re-set the galvanometer zero control to obtain a zero reading. Add the E.D.T.A. in 0-02 ml. or 0-01 ml. quantities. The end-point is determined as above. This can be interpolated to within 0-01 or 0-005 ml. if 0-02 ml. or 0-01 ml. additions are used.

The titration reading should be 0-5 ml. of E.D.T.A. for 1 ml. of the dilute calcium standard after allowance for the blank reading. The E.D.T.A. can be adjusted or a titration factor applied.

DETERMINATION OF REAGENT BLANK Using analytical reagents the blank values are extremely low (approximately 0-005 ml.) and to evaluate them it is necessary to follow Wilkinson's (1957) method of titrating a standard solution to past the end-point, adding more standard to the beaker and re-titrating.

Titrates 2 — titration 1 = true reading of standard

Titrates 1 — true standard reading = blank reading

TITRATION OF SERUM Into the 4 ml. beaker pipette 0-5 ml. serum and 0-5 ml. 2N NaOH and 0-2 ml. dilute calcon indicator and approximately 0-5 ml. of distilled water to adjust the level of the solution above the light path. Titrate as above using increased or maximum sensitivity for lipaemic or coloured sera.

When necessary satisfactory results can be obtained on 0-2 ml. of serum using titration additions of 0-01 ml. E.D.T.A.

CALCULATION Using 0-5 ml. serum calcium (mg./100 ml.)

\[ \text{titration reading} \times 0.1 \times 2 \]

STORAGE OF CALCON SOLUTION Satisfactory results were obtained with stock calcon and dilute calcon solution stored at room temperature up to a period of eight weeks and three weeks respectively. Storage in a deep freeze cabinet or refrigerator considerably increased the period of stability.

OPTIMUM CALCON CONCENTRATION It was found that the optimum calcon concentration was that which gives a free dye extinction of about 0-2 to 0-3 at 490 mµ. Using the indicator specified above this corresponds to 0-2 ml. dilute indicator as in the method described. The indicators obtained from British Drug Houses and Hopkins and Williams had to be made up to double the concentration indicated. These indicators appear to give an inferior visual end-point but can be used with the titrimeter.

RESULTS RECOVERY OF CALCIUM ADDED TO SERUM Varying amounts of calcium were added to three normal sera by adding stock calcium standard to the titration beaker after pipetting the serum. Table I indicates

---

FIG. 1. Titration curve for dilute calcium standard with E.D.T.A. using calcon as indicator.
the satisfactory recoveries obtained. Similar recovery experiments were carried out on lipaemic, haemolysed, and icteric sera. In such cases visual colour change varies according to the colour of the original serum, and, in most cases, the galanometer sensitivity setting should be increased to give satisfactory deflection readings. In cases of gross lipaemia the maximum sensitivity setting should be used. Table I indicates satisfactory recoveries in all cases investigated.

**EFFECT OF MAGNESIUM** Magnesium is precipitated at the pH used in the method, and it was considered possible that calcium might be co-precipitated or that the precipitate formed might interfere with the end-point. Varying amounts of stock magnesium standard were therefore added to the titration beaker after pipetting the serum or calcium solution. Table II shows satisfactory calcium recoveries even at levels of 100 mg.% magnesium. However, the heavy flocculant precipitates at high magnesium concentrations above 20 mg.% cause jumpy galvanometer readings due to interference with the light beam. Such values, however, are unlikely to be found in pathological sera.

**EFFECT OF PHOSPHATE** It was expected that high levels of phosphate would interfere with the end-point. Phosphate was therefore added to serum in the titration beaker using varying amounts of a solution of potassium dihydrogen phosphate equivalent to 50 mg. P/100 ml. Table III shows the results obtained. The interference with the end-point is characterized by a premature appearance of the blue end-point which then slowly reverts back to red. By slow titration this effect can be minimized up to a
phosphate level of 10 mg./100 ml. Bett and Fraser (1959) noted that the interference was directly related to the concentration of phosphate in the titration solution and not to the ratio of the calcium and phosphate ions. Thus by further dilution of the titration solution with 1 ml. or 2 ml. of water the level at which phosphate interferes can be raised to 15 to 20 mg./100 ml. If this dilution is carried out a corresponding increase in the amount of calcon dye should be made. Since 2N NaOH is used to give the high pH for the titration, the dilution can be made without going below pH 13. However, if many estimations are to be carried out on materials with a high phosphate content it is desirable to use a morpholine nitrate-nitric acid precipitation technique discussed below.

**EFFECT OF ANTICOAGULANTS** Calcium concentrations were estimated on sera obtained from normally clotted blood and on plasma samples obtained by use of E.D.T.A. oxalate, heparin, and liquid citrate as anticoagulants.

The E.D.T.A. plasma gave zero values. Oxalated plasma appeared to retain a very small amount of calcium which approximated to 0-4 mg./100 ml. in all samples. Using liquid citrate as anticoagulant and allowing for dilution, calcium results were obtained which were very closely comparable with those on sera (mean difference = + 0-10 mg./100 ml. ± 0-009). Heparinized plasma also gave results which compared very closely with those found in sera from the same subjects (mean difference + 0-02 mg./100 ml. ± 0-003).

Calcium estimations by the Clark and Collip (1925) oxalate precipitation method (modified by Wheatley, 1944) and the calcon method described were made on 55 miscellaneous normal and pathological sera with the following results. The oxalate precipitation method gave a mean on 55 sera of 9-85 mg./100 ml. ± 1-26 (S.D.) and that of the calcon/E.D.T.A. method 9-70 mg./100 ml. ± 1-24 (S.D.). This gave a mean difference of + 0-15 mg./100 ml. ± 0-029

### TABLE II

**EFFECT OF ADDED MAGNESIUM ON CALCIUM ESTIMATION**

<table>
<thead>
<tr>
<th>Magnesium Added (mg./100 ml.)</th>
<th>Calcium Found (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>10-0</td>
</tr>
<tr>
<td>5-0</td>
<td>10-0</td>
</tr>
<tr>
<td>10-0</td>
<td>10-0</td>
</tr>
<tr>
<td>20-0</td>
<td>10-0</td>
</tr>
<tr>
<td>50-0</td>
<td>9-8</td>
</tr>
<tr>
<td>100-0</td>
<td>9-8</td>
</tr>
<tr>
<td>Serum I</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>9-4</td>
</tr>
<tr>
<td>5-0</td>
<td>9-4</td>
</tr>
<tr>
<td>10-0</td>
<td>9-4</td>
</tr>
<tr>
<td>20-0</td>
<td>9-4</td>
</tr>
<tr>
<td>Serum II</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>10-0</td>
</tr>
<tr>
<td>10-0</td>
<td>10-0</td>
</tr>
<tr>
<td>100-0</td>
<td>10-2</td>
</tr>
<tr>
<td>Serum III</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>10-4</td>
</tr>
<tr>
<td>5-0</td>
<td>10-4</td>
</tr>
<tr>
<td>50-0</td>
<td>10-6</td>
</tr>
<tr>
<td>Serum IV</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>10-2</td>
</tr>
<tr>
<td>5-0</td>
<td>10-2</td>
</tr>
<tr>
<td>10-0</td>
<td>10-4</td>
</tr>
<tr>
<td>20-0</td>
<td>10-2</td>
</tr>
</tbody>
</table>

### TABLE III

**EFFECT OF ADDED PHOSPHATE ON CALCIUM ESTIMATION**

<table>
<thead>
<tr>
<th>TotalPhosphate Present (mg. P/100 ml.)</th>
<th>Calcium Found (mg./100 ml.)</th>
<th>Effect on End-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-7</td>
<td>9-4</td>
<td>No interference</td>
</tr>
<tr>
<td>5-7</td>
<td>9-4</td>
<td>No interference</td>
</tr>
<tr>
<td>8-7</td>
<td>9-4</td>
<td>No interference</td>
</tr>
<tr>
<td>13-7</td>
<td>9-2</td>
<td>Moderate interference</td>
</tr>
<tr>
<td>18-7</td>
<td>9-0</td>
<td>Definite interference</td>
</tr>
<tr>
<td>23-7</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>53-7</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>Serum II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-2</td>
<td>9-2</td>
<td>No interference</td>
</tr>
<tr>
<td>6-2</td>
<td>9-2</td>
<td>No interference</td>
</tr>
<tr>
<td>9-2</td>
<td>9-2</td>
<td>Slight interference</td>
</tr>
<tr>
<td>12-2</td>
<td>9-2</td>
<td>Moderate interference</td>
</tr>
<tr>
<td>14-2</td>
<td>9-2</td>
<td>Definite interference</td>
</tr>
<tr>
<td>19-2</td>
<td>9-0</td>
<td>Gross interference</td>
</tr>
<tr>
<td>24-2</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>Standard calcium solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10-0</td>
<td>No interference</td>
</tr>
<tr>
<td>3-0</td>
<td>10-0</td>
<td>No interference</td>
</tr>
<tr>
<td>5-0</td>
<td>10-0</td>
<td>Very slight interference</td>
</tr>
<tr>
<td>11-0</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>20-0</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>50-0</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
<tr>
<td>100-0</td>
<td>No accurate result</td>
<td>Gross interference</td>
</tr>
</tbody>
</table>
(S.E.M.). The Student t test gave \( p = < 0.001 \). Thus there is a small but significant difference between the mean values of the two series, indicating that the E.D.T.A. results are slightly lower than those obtained with the oxalate precipitation method. However, the difference does not appear to be as great as that reported for other E.D.T.A. titrations by Wilkinson (1957) and Golby et al. (1957).

**CALCIUM ESTIMATIONS ON BIOLOGICAL SPECIMENS USING MORPHOLINE NITRATE-NITRIC ACID EXTRACTS**

Horner (1955) and later Baron and Bell (1959) described a quick method for preparing protein and phosphate-free extracts from biological samples without the preliminary ashing or precipitation of calcium. Phosphates and proteins are directly precipitated with a morpholine nitrate-nitric acid reagent and sodium tungstate leaving a clear acid supernatant which, by the addition of the normal quantity of NaOH, is ready for immediate titration with calcon and E.D.T.A.

**REAGENTS FOR PRECIPITATION** Two are required:—

1. **Morpholine nitrate-nitric acid reagent** (Horner, 1955; Baron and Bell, 1959) A 1:1 mixture of 25% v/v HNO₃ and 48% morpholine nitrate. 48% morpholine nitrate is made by adding 50% HNO₃ to 28 ml. morpholine until the solution is neutral followed by dilution with water to 100 ml. We found that it was essential to add 50% HNO₃ very slowly to morpholine cooled below 0°C. If the temperature is allowed to rise above 5°C the resultant reagent may result in discoloration of the calcon giving poor end-points and high blank values.

2. **Sodium tungstate** 66% w/v Na₅WO₄2H₂O

**MORPHOLINE NITRATE PRECIPITATION** Two millilitres of the sample, prepared to contain approximately 20 mg Ca/100 ml., 1 ml. sodium tungstate, and 2 ml. morpholine nitrate-nitric acid reagent are mixed thoroughly, allowed to stand for one hour at room temperature, and centrifuged for 10 minutes at 3,000 r.p.m.

**TITRATION** Pipette 1 ml. supernatant, 0.5 ml. 2N NaOH, and 0.2 ml. dilute calcon into a titration beaker and titrate as previously described.

\[ \text{mg Ca/100 ml. prepared sample} = \text{test} - \text{blank} \times 10 \times 2.5 \]

**RESULTS WITH URINE** Direct titrations are possible on urine (0.5 ml. or 0.2 ml.) with low phosphate concentrations but may become rather difficult on urine with large concentrations of phosphate. The calcium content of 23 urines was therefore estimated by the morpholine nitrate method described and for comparison by preliminary oxalate precipitation of calcium followed by titrations with E.D.T.A. and calcon. With this method 1 ml. of 4% ammonium oxalate was added to 2 ml. of urine (pH 5) and 2 ml. of water. After mixing and leaving for one hour the precipitate was centrifuged for 10 minutes at 3,000 r.p.m., the supernatant decanted off, and the precipitate dissolved in 0.5 ml. 2N HCl and made up to 4 ml. with water. One millilitre of solution (= 0.5 ml. urine) was titrated with E.D.T.A. and calcon. Figure 2 gives the results obtained and shows good agreement between the two methods. The morpholine nitrate method is a slightly quicker and probably more accurate method as it eliminates the need for the actual precipitation of calcium.

**RESULTS WITH MILK AND FOOD** The calcium content of milk and food was estimated by the rather laborious method of ashing followed by titration of the calcium present with E.D.T.A. and calcon and by direct morpholine nitrate precipitation followed by titration of the supernatant produced with E.D.T.A. and calcon. The good agreement between the two methods shown in Table IV indicates that the second rapid method is satisfactory.

**RESULTS WITH SERUM** The calcium content of 12 sera was estimated by direct titration with E.D.T.A. and calcon and by the morpholine nitrate method described. Figure 4 shows that the results are closely comparable but it has been found unnecessary to use this longer method for routine work. However, in
TABLE IV
ESTIMATION OF CALCIUM IN MILK AND FOOD BY TWO METHODS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Estimation after Morpholine Nitrate Ashing (a)</th>
<th>Estimation after Morpholine Nitrate Precipitations (b)</th>
<th>Difference between (a) and (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (mg./100 ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>120</td>
<td>127</td>
<td>- 6</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>128</td>
<td>112</td>
<td>+18</td>
</tr>
<tr>
<td></td>
<td>133</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Food (mg. of total diet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>660</td>
<td>635</td>
<td>+16</td>
</tr>
<tr>
<td></td>
<td>660</td>
<td>652</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>700</td>
<td>720</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>742</td>
<td>720</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>375</td>
<td>395</td>
<td>-20</td>
</tr>
</tbody>
</table>

cases with grossly raised phosphate levels the endpoints obtained are improved.

ESTIMATIONS OF TOTAL MAGNESIUM AND CALCIUM IN SERUM

Using the apparatus described the magnesium content of serum may also be estimated by first titrating the total calcium and magnesium present with E.D.T.A. at a pH of approximately 10-5 with Eriochrome black T indicator followed by titration of calcium only at a pH of approximately 13 with E.D.T.A. and calcon as previously described. For each titration 0-5 ml. of serum is required.

The reagents used for total magnesium and calcium estimations are basically the same as described by Wilkinson (1957) but adjusted to suit the smaller volumes required for the apparatus by using 1 ml. of Eriochrome dye buffer solution which had the dye concentration reduced by 40%.

REAGENTS These are as follows:

Stock magnesium standard (50 mg./100 ml.) MgSO₄·7H₂O, 2·5328 g., is dissolved in 500 ml. ion exchange distilled water.

Dilute magnesium standard (5 mg./100 ml.). Dilute stock solution 1:10 with distilled water.

Ethanolamine (2 hydroxethanolamine, B.P. 173°C.) It may be necessary to re-distil this reagent.

Methyl alcohol absolute AR

Eriochrome black T stock dye solution Dissolve 400 mg. Eriochrome black T (G. T. Gurr Ltd.) in 100 ml. methyl alcohol, add 4 ml. ammonia AR 0·88 SG solution, and store in a polythene bottle. The dye is stable up to five or six months.

Dye buffer solution Eriochrome dye, 0·5 ml., and 0·5 ml. ethanolamine are made up to 50 ml. with distilled water. The dye buffer solution is made freshly each day.

METHOD The use of the apparatus is the same as described for the estimation of calcon. The Eriochrome black T colour change is sharper than that of calcon and the change of optical density is greater, so that the sensitivity settings of the galvanometer must be lowered.

CHECK ON E.D.T.A. STANDARDIZATION Into the titration beaker pipette 1 ml. or 1·2 ml. magnesium dilute standard and add 2 ml. of dye buffer solution. Titrate with E.D.T.A. until the full colour change has been observed and the E.D.T.A. titration figure giving the greatest galvanometer deflection noted.

1 ml. magnesium standard = 0·833 ml. E.D.T.A.
1·2 ml. magnesium standard = 1 ml. E.D.T.A.

It is useful to cross check the E.D.T.A. solution both with the magnesium standard and Eriochrome black T and with the calcium standard and calcon. After allowing for blank values there should be complete agreement of standardization.

TITRATION OF TOTAL CALCIUM AND MAGNESIUM IN SERUM

Into the titration beaker pipette 0·5 ml. serum, add 1 ml. dye buffer solution, and titrate as before. Maximum accuracy is obtained using 0·01 additions of E.D.T.A. The pH of the dye buffer and serum is approximately 10·5.

Calcium only is estimated on a further 0·5 ml. of serum as described previously.

CALCULATION The titration for magnesium equals the total calcium and magnesium titre minus the titre for calcium only. [Mg (mg./100 ml.) = titre Mg × 2 × 5/standard reading (1 ml.).]

RECOVERY OF MAGNESIUM ADDED TO SERUM Varying quantities of magnesium sulphate solution (as 50 mg.
C. H. Bowden and Valerie J. Patston

Table V shows that the method gives satisfactory recoveries up to 20 mg. Mg/100 ml.

We wish to thank Professor N. F. Maclagan for his interest and help while these experiments were in progress.

REFERENCES


The November 1962 Issue

THE NOVEMBER 1962 ISSUE CONTAINS THE FOLLOWING PAPERS

Haemagglutination in acute hepatitis and other diseases
PAUL TURNER, V. N. JHA, NUALA CROWLEY, and SHEILA SHERLOCK

An epidemiological study of haemagglutination in hepatitis
JAMES R. MCARTHUR and NUALA CROWLEY

Blood clotting factors in cerebrospinal fluid
STEFAN NIEWIAROWSKI, IRENA HAUSMANOWA-PETRUSEWICZ, and
ZENON WEGRZYNOWICZ

Observations on the separate assessment of Prower-Stuart factor activities
C. GARDIKAS, C. LYBERATOS, G. KALLINIKOS, and M. KALLINIKOU

An investigation into the use of activated and non-activated papain in routine rhesus blood grouping
G. C. B. WINTER and B. E. WRATTEN

The contact phase of coagulation in the presence of heparin
A. L. BLOOM

Muscle morphology in infantile protein malnutrition
R. D. MONTGOMERY

The diagnosis of the scars of chronic pyelonephritis
J. F. SMITH

Laryngeal dysfunction and the pulmonary syndrome of the newborn
G. R. OSBORN and R. L. FLETT

Haematoxylin bodies in Hodgkin's disease
N. CANDREVIOTIS

Resistance of Salmonella typhi to chloramphenicol
B. RAMANARAYANA MURTI, K. RAJYALAKSHMI, and C. S. BHASKARAN

Part I A preliminary report
Part II The mechanism of resistance

The dispersal of staphylococci in hospital wards
W. C. NOBLE

The dispersal of organisms from minor septic lesions
B. T. THOM and R. G. WHITE

Serum tube identification of Candida albicans
D. W. R. MACKENZIE

A simple colorimetric method for the determination of serum alpha-hydroxybutyric dehydrogenase activity
S. B. ROSALKI

The incidence of cryoglobulinaemia as determined by a turbidimetric method
L. BAGRATUNI

The determination of urinary 17-ketosteroids by an improved Zimmermann reaction
R. N. BEALE, J. O. BOSTROM, and D. CROFT

Blood pyruvate concentration measured by a specific method in control subjects
J. LANDON, J. K. FAWCETT, and VICTOR WYNN

The estimation of two alpha, glycoproteins (orosomucoid and another alpha, acid glycoprotein) in health and disease
J. A. EASTON, J. HARDWICKE, and P. H. WHITEHEAD

Technical methods
An improved container for cells preserved by freezing
J. H. L. PLAYFAIR and D. E. PEGG

Macroscopic demonstration of infarction in fresh brain slices
D. N. Raine

A routine immunofluorescence method for detecting autoantibodies to thyroid colloid
R. C. NAIRN, T. GHOSE, I. B. PORTEOUS, and J. A. URQUHART

Book reviews
Index to volume 15

Copies are still available and may be obtained from the PUBLISHING MANAGER.

BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, W.C.1, price 17s. 6d.
Microdetermination of calcium and magnesium in biological materials
C. H. Bowden and Valerie J. Patston

J Clin Pathol 1963 16: 18-24
doi: 10.1136/jcp.16.1.18

Updated information and services can be found at:
http://jcp.bmj.com/content/16/1/18

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/