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BIOCHEMICAL ESTIMATIONS USING MICRO QUANTITIES

MISS L. JONES
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INTRODUCTION

The difficulty of venipuncture in babies and young children is well known; paediatric hospitals overseas use micro techniques for blood analysis.

After thorough checking against the corresponding macro biochemical tests, methods involving 0.1 ml. quantities of whole blood, plasma or serum, are being carried out at Auckland Hospital. Although these methods are still under trial and improvements will no doubt result from further experience, the estimations, carried out both on small blood samples and on capillary blood samples, have proved accurate, and the technique for the collection of the latter samples has proved simple and satisfactory.

GENERAL TECHNIQUE

1. *Collection of Capillary Blood Samples*

The blood sample is taken from a finger, heel or ear prick into a capillary tube which is then sealed with sealing wax.

2. *Preparation of capillary collecting tubes*

Capillary tubing with an internal diameter of 1.5-2.0 mm. and an external diameter of 2.5-3.5 mm. is used. The tubing is drawn out to form a fairly fine tip, then cut to form a collecting tube 10-18 cm. in length, depending on the bore of the tubing used. 0.2 ml. of blood should be contained in approximately three-quarters of the length of the tube. The tubes are dichromated, then washed thoroughly and dried in a hot air oven.

3. *Anticoagulants*

(a) Heparin: A solution containing 1 mgm. heparin/ml. is used.

(b) Oxalate: A 2.5% solution of potassium oxalate is used.

The anticoagulant solution is drawn up and down the collecting tube several times, then the tubes are placed in a horizontal position to dry in a hot air oven. In practice, almost all estimations are carried out on serum.

4. *Waxing of Tubes*

A hard sealing wax (post office red wax) is heated. The wider end of the collecting tube is then heated and rotated in the wax to form an even coating about 5 mm. in length around the end of the tube but without sealing the tube.

5. *Collection of Specimens*

A rubber suction tube is attached to the rim of wax. The rubber tubing normally used for this purpose may be made to fit

the waxed collecting tube, by folding back the tip to form a lip, or by fitting into it a short piece of glass tubing which has been drawn out at one end to allow a narrower piece of rubber tubing to be attached. Blood from a finger, heel or ear prick is drawn up into the collecting tube to about three-quarters of its length. An efficient pricker and a good circulation in the area to be pricked are essential. A broad bevelled-edge pricker producing a very small cut was found more efficient than a fine long pointed one, and with only a little experience several collecting tubes may be filled without difficulty from one prick. In the case of an infant with a high haemoglobin concentration more than 0.2 ml. of blood should be collected to ensure obtaining 0.1 ml. serum. The waxed end of the collecting tube is then thoroughly sealed by rotating it in heated sealing wax. The tube itself should only be heated sufficiently to ensure a satisfactory seal. A spirit burner is a convenient source of heat.

6. *Separation of Serum or Plasma*

The sealed collecting tube should be broken about 1 cm. from the drawn out tip so that the serum is contained in a piece of tubing of uniform diameter. This is advisable as the narrow tip may become partially blocked with blood and this results in the failure of the serum to separate satisfactorily on centrifuging, and the entry of air bubbles into the graduated pipette when transference is attempted.

The capillary tube is then placed in a test tube and centrifuged at 2,500 r.p.m. for 5-10 minutes.

The tube is broken at the interface of cells and serum by marking with a glass file at this point and breaking.

7. *Transference of Serum or Plasma to Graduated Micro Pipette*

The capillary tubing containing the serum or plasma is held horizontally and the tip of a clean, dry graduated 0.1 ml. pipette with rubber suction tube attached is placed in contact with it. The serum is then gently drawn up into the graduated pipette.

The equipment required to be taken to the wards for the collection of capillary blood samples is easily compacted in a "collect box." The box was designed to contain:—

1. Three drawers, holding cotton wool swabs, sealing wax, matches, collecting tubes, graduated 0.1 ml. pipette.

2. A sliding perspex rack with holes provided for

(a) test tubes to hold the sealed collecting tubes,

(b) corked N.P.N. tubes,

(c) distilled water, S.V.R., ether and paraffin,

(d) a small spirit burner,

(e) small tubes containing prickers.

PROCEDURES

Most methods are a simple scaling down of established general laboratory procedures.

1. *SERUM SODIUM AND POTASSIUM*

A 1 in 40 serum dilution is made using 0.1 ml. serum for potassium and from this dilution a 1 in 400 dilution for sodium is made. The Na and K concentrations are determined in the E.E.L. flame photometer, set at 100% transmission with a 1 mgm./100 ml. standard.

2. *FLOCCULATION TESTS OF LIVER FUNCTION*

The quantities used are half those used in the general procedures.

(a) *Zinc Sulphate Turbidity.* 0.05 ml. serum in 3.0 ml. of zinc sulphate buffer. The optical density is read after 30 minutes at 650 mu. in the Unicam S.P. 600 spectrophotometer.[1]

(b) *Thymol Turbidity.* 0.04 ml. serum in 2.4 ml. thymol reagent. The optical density is read at 700 mu. as above.[2]

(c) *Colloidal Gold.* As done routinely, using 0.05 ml. serum.[3]

3. *ALKALINE PHOSPHATASE*

A scaling down of King's method[4] using 0.05 ml. serum for test and blank, in 2.0 ml. of buffered substrate. After incubation 0.95 ml. of dilute (1:3) Folin-Ciocalteau phenol reagent is added, and the solutions mixed and centrifuged.

Colour Development. 2.0 ml. of the supernatant from both test and control solutions are pipetted into test tubes. Into a third tube is pipetted 1.0 ml. of the standard phenol solution (without reagent), 0.6 ml. of Folin-Ciocalteau reagent and 0.4 ml. water. 1.0 ml. of 15% sodium carbonate is added to each tube and the three tubes placed in the 37° C. bath for 10 minutes. The optical densities of test, control and standard are read at 760 mu.

$$\text{Then } \frac{\text{O.D. Test - O.D. Blank}}{\text{O.D. Standard}} \times 30 = \text{Phosphatase units.}$$

Stock Standard Phenol. Prepare by dissolving 0.3 gm. A.R. phenol in 250 ml. 0.1N HC1.

A working solution of standard phenol (without reagent) is prepared by adding 1.0 ml. of stock standard phenol to a 100 ml. volumetric flask and diluting with distilled water to 100 ml.

4. *CHOLESTEROL*

0.1 ml. serum, plasma or whole blood, is added to extracting fluid (alcohol/ether) and proceed as in the King modification[5] of the method of Sackett using half quantities throughout, and reading the optical densities in the spectrophotometer at 650 mu. The

cholesterol value in mgm./100 ml. is read directly from a calibration graph, prepared in the following way:—

0-2.5 ml. quantities of the working solution are diluted to 2.5 ml. with chloroform. 1.0 ml. acetic anhydride and 4 drops of concentrated sulphuric acid are added. The solutions are well mixed and allowed to stand in the dark 10 minutes. They are read in the Unicam at 650 mu. and the optical density/concentration graph is plotted. 0-2.5 ml. quantities of working standard correspond to a range of 0-500 mgm. cholesterol/100 ml.

Standard Cholesterol Solutions

(a) Stock Solution: Exactly 0.80 gm. of pure cholesterol is dissolved in pure anhydrous chloroform and the volume made up to 100 ml. in a clean, dry volumetric flask. It is stored in a well-stoppered amber bottle.

(b) Working Solution: 2.5 ml. of the Stock Standard Solution are diluted to 100 mls. with chloroform in a dry volumetric flask.

5. *NON-PROTEIN NITROGEN*

A 1 in 40 Folin-Wu filtrate is prepared using 0.1 ml. of whole blood or serum. 1.0 ml. of this is digested and nesslerised.

6. *SERUM PROTEINS*

Total Protein: 1.0 ml. of a 1:100 serum dilution in water (using 0.05 ml. serum) is digested and nesslerised.

Albumin: 1.0 ml. of a 1:62 serum dilution in 22.2% sodium sulphate (using 0.05 ml. serum) is digested and nesslerised.

The total protein dilution is also used to determine the N.P.N. correction figure.

7. *GLUCOSE*

This test is routinely carried out on 0.1 ml. quantities.[6]

8. *BILIRUBIN*

A modification of Powell's method[7] is used. 0.05 ml. quantities of serum for test and for blank are pipetted directly into two cuvettes. 0.025 ml. of diazo test and blank solutions respectively are added, followed by 2.425 ml. of 10% sodium benzoate. After 5 minutes the optical densities are read at 525 mu. in the spectrophotometer, and bilirubin values are read directly from a calibration graph.

Solutions

(a) 10% Sodium Benzoate: dissolved in distilled water and filtered.

(b) Solution A: one gram of sulphanilic acid is dissolved in a little water containing 15 ml. of concentrated HC1 and the solution is made up to one litre with distilled water.

(c) Solution B: 0.5% sodium nitrite in distilled water.

(d) Ehrlich's Diazo Reagent: freshly prepared before use by adding 10 ml. Solution A to 0.6 ml. Solution B.

(e) Diazo Blank: 15 ml. of concentrated HC1 in one litre of distilled water. 10 ml. of this solution are added to 0.6 ml. of Solution B.

Bilirubin Standard for Calibration Graph

10.0 mgm. of bilirubin are dissolved in 10 ml. of 1% Na_2CO_3. This is best carried out in a weighing bottle and is aided by holding the bottle under the hot tap. The contents of the bottle are then transferred, with rinsing to a 50 ml. volumetric flask containing 15 ml. of protein solution, and the pH adjusted to 7.4 with acid phosphate solution (about 0.6 ml. of 29% $NaH_2PO_4 . 2H_2O$). The volume is then made up to 50 ml. with protein solution. This standard is equivalent to 20 mgm. bilirubin/100 ml. and dilutions of it are made to give a range of 0-20 mgm./100 ml. for the construction of a calibration graph. The standard must be kept in the dark.

Note: At this dilution (1:50) the accuracy is unavoidably low with bilirubin values in the normal and moderately raised range. However, the primary purpose of this micro method is to assist in deciding when replacement transfusion is necessary in Rh babies, in whom bilirubin levels are always high.

9. *UREA*

0.1 ml. of whole blood or plasma in isotonic sodium sulphate is incubated with urease, and the ammonia produced estimated by nesslerisation, as in King's method[8] using half quantities. Stabilising agents, sodium potassium tartrate and potassium persulphate[9] are added before nesslerisation, each in proportion of 15% of the final nesslerised volume. A urease blank is prepared similarly to the blood sample and subtracted from the final result. The optical densities are read at 460 mu.

Solutions

(a) Sodium Potassium Tartrate solution: 1%.

(b) Potassium Persulphate: 2.5%.

10. *PHOSPHORUS*

A modification of Horwitt's method is used.[10]

0.1 ml. quantities of serum or plasma are precipitated in twice the total volume of trichloracetic acid used by Horwitt. The concentration of acid was changed from 15% to 10% to eliminate the necessity for two different acid solutions.

The colour development is identical with Horwitt's, except that relatively twice the amount of supernatant fluid is used in pro-

portion to the other reagents. No time interval is allowed between the additions of ammonium molybdate and stannous chloride as this was found to cause no appreciable difference to the final optical density readings.

A blank is treated similarly and the optical densities are read in the Unicam spectrophotometer at 700 mu. These are converted into mgm. of inorganic phosphate/100 ml. from a calibration curve prepared by estimating the phosphorus concentrations of serial dilutions of a 10 mgm./100 ml. phosphorus standard.

11. *CHLORIDES*

Capillary blood samples are collected in collecting tubes not under paraffin. It was found that the chloride content of non-paraffin capillary tube samples does not differ appreciably from that of paraffin samples over a period of 24 hours.

The method of Sendroy[11] modified by Hiller[12] is employed, using 0.1 ml. of serum or plasma. This is pipetted into 1.5 ml. of water and 1.0 ml. of phosphoric-tungstic acid solution is added. 30 mgm. of pulverised silver iodate are added, the tube stoppered, shaken vigorously for 40 seconds, then centrifuged.

1.0 ml. of the clear supernatant and 100 mgm. of solid potassium iodide are titrated with 0.00411 N. sodium thiosulphate delivered from a 10 ml. microburette using starch as indicator.

The end point is a very sensitive one. The chloride content of the sample is calculated from the amount of thiosulphate used in the titration.

ml. of thiosulphate used X 100 = mgm./100 ml. chloride in serum or plasma expressed as sodium chloride.

Note

1. The test sample is pipetted into water. To compensate for this the strength of the phosphoric-tungstic acid solution was increased; viz. 15 gm. $Na_2WO_4.2H_2O$ dissolved in 900 ml. of water, and 25 ml. concentrated H_3PO_4. The volume is made up to a litre with water.

2. 0.1% borax is added to both stock and standardised thiosulphate as stabiliser.

12. *CO_2 CONTENT*

Collection of Specimens: The capillary blood sample is taken into a collecting tube in such a way that it is exposed to the air as little as possible. To ensure this, a small amount of paraffin is first drawn up into the tube, then the blood is sucked up as soon as it emerges from the puncture wound, taking care to avoid entrapping air bubbles. When sufficient blood has been taken up, a further small amount of paraffin is drawn up to seal off the column from the air.

Determination: The CO_2 content of the serum sample is measured with the manometric Van Slyke apparatus, using 0.1 ml. of serum[13]. The CO_2 content of the peripheral blood is approximately four volumes per cent. lower than the CO_2 combining power because in the latter estimation the serum is equilibrated with alveolar air at a lower temperature.

13. *CALCIUM*

The method of Rehell[14] slightly modified, is used. Ammonium oxalate is added to 0.1 ml. serum and the precipitated calcium oxalate dissolved in hydrochloric acid. 0.1 ml. of ethylene diamine tetra acetate is added and the solution made alkaline with 1 ml. of ammonia buffer. The solution is titrated with magnesium chloride delivered from a Conway microburette, using the dye Eriochrome Black T as indicator. The titration figure is converted into mgm. Ca per 100 ml. frcm a standard curve.

Note

1. It was found that a more alkaline pH resulted in an improved end point. The strength of the buffer solution was increased (170 ml. concentrated ammonia made up to 1,000 ml.) resulting in a final pH of 10.2.

2. Eriochrome Black T alone (0.5% in methyl alcohol) is used as indicator, as the mixture Eriochrome Black T + Methyl Red + hydroxylamine hydrochloride was found to be unstable, fading rapidly.

3. The full colour change involves a gradual change from blue through purple to a red colour, and the use of an artificial matching standard is essential for accuracy.

End Point Colour

10 ml. S.V.R.
2 drops 0.2N NaOH.
0.026 ml. Brom-cresol Purple (0.02 gm. in 10 ml. S.V.R.).
0.0114 ml. Congo Red (50 mgm., 9 ml. H_2O, 1 ml. alcohol).
0.04 ml. Sudan Black (0.01 gm. in 10 ml. S.V.R.).

This solution is unstable, and should be freshly made up if fading is suspected.

The test solution is placed in a small rack beside the standard. The end point chosen is a blue-purple with a tinge of red as (i) this is not the first colour change observed and definite warning that the end point is approaching is given and (ii) the point when a very small excess of magnesium solution has been added is readily detected.

DISCUSSION

Details of the above methods are not included as they are not new and are available in the references given.

The accuracy of all methods was checked against determinations carried out on a macro scale and there was found to be good agreement between the two. A Unicam SP 600 spectrophotometer is used at present at this hospital, with cuvettes of minimum volume 2.5 ml.

Although the methods are primarily intended for use in the paediatric wards, they have proved useful in general cases where venipuncture is difficult or where only a very small sample is obtained.

ACKNOWLEDGEMENT

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REFERENCES

1. Kunkel, H. G., Proc. Soc. exp. Biol. and Med., **66**, 217, 1947.
2. Maclagan, Brit J. exp. Path., **25**, 234, 1944.
3. Maclagan, Ibid., **25**, 15, 1944.
4. King, Microanalysis in Medical Biochemistry (2nd Edn.), 1951, p. 69.
5. King, Ibid. (2nd Edn.), 1951, p. 39.
6. King, Ibid. (2nd Edn.), 1951, p. 23.
7. Powell, Am. J. clin. Path., **14**, 55, 1944.
8. King, Microanalysis in Medical Biochemistry (2nd Edn.), 1951, p. 8.
9. Guntzkow, J. Biol. Chem. **143**, 531, 1943.
10. Horwitt, Ibid., **199**, 537, 1952.
11. Sendroy, Ibid., **120**, 335, 405, 419, 1937.
12. Hiller, Practical Clinical Chemistry, 1953, p. 78.
13. Peters and Van Slyke, Quantitative Clinical Chemistry, Vol. II, Methods, 1932, p. 283.
14. Rehell, Scand. J. clin. and Lab. Invest., **6**, 335, 1954.

INVESTIGATION OF HAEMOLYTIC TRANSFUSION REACTION

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Haemolytic transfusion reaction may be defined as occurrence of increased rate of destruction of the red cells of either the donor or recipient following transfusion. Blood destruction may be:

1 *Intravascular*—rupture of corpuscles in bloodstream with consequent liberation of haemoglobin into plasma.

2. *Extravascular*—disappearance of corpuscles from circulation without liberation of haemoglobin into blood stream—this commonly occurs with transfusion of over-aged stored blood.

Intravascular haemolysis demonstrable in transfusion reactions may be due to:

1. Result of destruction of injected red cells by the iso-antibody of the recipient.

ABO group—blood cannot be transfused from those donors whose isoagglutinogen can react with the agglutinins in the blood of the recipient.

Rh Group and others—intra-group haemolytic reactions may occur if Rh. positive blood is given to Rh. negative recipients; this affects mainly recipients previously sensitized by transfusion and/or pregnancy. Sensitization to the E or C antigen may also occur.

Similar difficulty may arise with recipients sensitized to the c antigen, the Kell and the Duffy factors, but are much rarer.

2. Result of destruction of red cells of the recipient by the antibody of the injected blood.

Dangerous universal donors—blood containing the isoagglutinins anti-A or anti-B of high titre or present in the form of immune isoagglutinins must not be given to a recipient of group A, B or AB.

3. Transfused blood containing large amounts of free haemoglobin or excessively fragile cells, these being due to inadequately preserved blood, blood being stored at an unsuitable temperature or in solution not containing glucose, or over-aged blood.

Bacterial contamination will also produce signs and symptoms similar to haemolytic transfusion reaction.

Even with best techniques and management, transfusion reaction may occur due to:

1. Antibodies involved at time of transfusion are not detectable.
2. Difficulty to detect antibodies with methods available.

Haemolytic transfusion reactions in most cases are characteristic, however there are at least three reasons that justify preoccupation with this subject.

1. Cases in which the clinical manifestations are obscured by underlying disease.
2. Characteristic clinical picture of haemolytic transfusion is not always fully developed.
3. Some clinicians have a tendency to blame on transfusions all complications of disease following transfusion.

Fortunately laboratory tests may help to establish correct diagnosis providing they are employed properly and at the proper time. In at least three situations the aid of the laboratory is essential for correct diagnosis of haemolytic transfusion reactions.

1. Asymptomatic haemolytic transfusion reaction.
2. Haemolytic transfusion reaction simulated by the underlying disease.
3. Haemolytic transfusion reaction obscured by underlying disease.

Clinical symptoms as typical anginal precordial pain, severe lumbar distress and chill, followed by drop in blood pressure are frequently first indications of haemolytic process, but absence of these signs does not exclude haemolytic reaction. The following conditions may also belong: progressive renal failure (which may be part of the transfusion reaction, but also may be part of the underlying disease), acute liver insufficiency with jaundice, acute anaemia, haemoglobinuric nephrosis (due to causes other than transfusion—haemolysis and resulting from shock or haemorrhage), jaundice (following massive pulmonary infarction). Intravascular haemolysis may be responsible for various clinical manifestations besides being due to haemolytic transfusion reactions.

INVESTIGATIONS

The following measures should be taken in order to make possible complete investigation of transfusion reactions if and when they occur.

1. Every pre-transfusion specimen must be saved for a period of at least ten days, together with pilot tube of blood that was cross-matched for recipient.
2. After administration of blood, the empty blood bottle should be returned to the Blood Bank unwashed and kept for twenty-four hours.
3. When a transfusion reaction is reported or suspected, a specimen of blood from the recipient must be immediately obtained.
4. Specimen of urine should also be obtained immediately, and additional specimens collected.

The following tests are carried out immediately.

1. *Regroup.*

(a) ABO and Rh. group both donor's pilot tube and the pre and post-transfusion specimens from the recipient.

(b) ABO and Rh. group the few drops of remaining blood in bottle to ensure that no confusion has taken place between identity of pilot tube and actual blood contained in the bottle.

(c) A direct Coombs test should be performed on the post-transfusion specimen, a positive result will be given if the antibody has coated the red cells of the donor.

2. *Compatibility tests.*

(a) Cross-matching tests are repeated with the pre and post-transfusion specimens using in both instances the saline and albumin compatibility tests as well as the indirect Coombs test as some antibodies are detected only by this method.

If all these tests are negative, but there is good reason to suspect a haemolytic transfusion reaction, a cross-matching test with enzyme-treated red cells of donors is performed.

(b) If group O blood has been given to Group A (or B) recipient, examine a fresh specimen of blood from the donor to decide whether or not the transfused agglutinins are of high titre, or are present in the dangerous form of "immune" isoagglutinins (by the haemolysin test).

(c) If incompatibility is confirmed on retesting, identification of the antibody should be made. Full genotyping of the recipient is carried out, and the recipient's serum examined against a panel of selected donors. Titre of the offending agglutinin should be low immediately after transfusion, rising 10-14 days later.

3. *Presence of free haemoglobin.*

(a) Examine pre and post-transfusion specimens after centrifugation for presence of free haemoglobin in serum by spectroscopical examination.

(b) If more than six hours have elapsed between time of transfusion and taking of specimen, Schumm's test should be performed, this being a more sensitive test for methaemalbumin and is chiefly of value in examining samples which do not contain haemoglobin visible to the naked eye.

(c) Serum bilirubin and urea nitrogen should be estimated on pre and post-transfusion specimens for any increase, and also repeated at a twenty-four hour interval.

4. *Urine.*

Test for free haemoglobin and examine microscopically for red cells and red cell casts.

5. *Other factors.*

If all above tests give negative results a haemolytic transfusion reaction can be excluded. Other factors to be investigated should be the possibility of haemolysis caused through inadequate preservation of blood (improper temperature of storage), over-aged blood or simultaneous administration with the blood of incompatible solutions such as distilled water or glucose and water. Therefore it is highly desirable that records of administration of blood contain a definite statement as to the solutions that have been administered with it, or have passed through the tubing either before or after administration of blood.

6. *Bacterial contamination.*

A portion of the remaining blood in the bottle should be studied for possible bacterial contamination.

REFERENCES

Davidsohn and Stern, Am. J. clin. Path., 25, 381, 1955.
Dunsford and Bromley. Techniques in Blood Grouping.
Mollison. Diseases of Blood in Clinical Medicine.
Whitby and Britton. Disorders of the Blood.

LABORATORY DIAGNOSIS OF VIRUS DISEASES

The demonstration or isolation of active virus requires the proper collection of appropriate material. The preservation of this material both en route to and in the laboratory is important. The inoculation of susceptible animals, fertilized eggs or suitable tissue cultures are procedures used in the isolation of a virus. Finally, the presence of a virus may be demonstrated by the appearance of characteristic histologic lesions, inclusion bodies, viral antigens or viral antibodies.

The table printed here was presented at the 1956 Bacteriologists' Conference by Mr A. M. Murphy, Virus Research Officer, Auckland Public Hospital.

VIRUS	*DISEASE*	*ISOLATION*				*SEROLOGY*	
		MATERIAL	*DAY*	*HOST*	*ROUTE*	*MATERIAL*	*METHOD*
INFLUENZA A. B or C	Influenza with or without Pneumonia	Throat Washing Lung (P.M.)	1 - 3	Eggs	Amniotic Cavity	Serum, acute and convalescent	Complement Fixation
PSITTACOSIS	Pneumonia	Sputum Blood	1 - 7	Eggs	Yolk Sac	Serum, acute and convalescent	Complement Fixation
Q FEVER	Pneumonia and fever	Sputum Blood	1 - 5	Eggs Guinea Pig	Yolk Sac I.P.	Serum, acute and convalescent	Complement Fixation
ADENOVIRUS (A.P.C.)	Acute Respiratory Disease Pharyngitis Kerato-conjunctivitis Pneumonia	Throat Washing	1 - 3?	Tissue Culture	-	Serum, acute and convalescent	Complement Fixation Virus neutralisation
	Primary Atypical Pneumonia	Throat Washing Sputum Blood	as	above		Serum, acute and convalescent	Complement Fixation Cold agglutinins Streptococcus M.G. agglutinins
MUMPS	Meningitis Parotitis	Saliva	1 - 4	Eggs	Amniotic Cavity	Serum, acute and convalescent A presumptive diagnosis can be given on examination of a single specimen collected after the 5th day.	Complement Fixation
LYMPHOCYTIC CHORIOMENINGITIS	Meningitis Pneumonia	C.S.F.	1 - 7	Mice Guinea Pigs	I.C. I.P.	Serum, acute and convalescent	Complement Fixation Virus neutralisation
POLIOVIRUS	Ant. Poliomyelitis Polioencephalitis	Faeces Brain and cord (P.M.)	1 - 8	Tissue Culture		Serum, acute and convalescent	*Complement Fixation
COXSACKIE							

	Encephalitis						
HERPES SIMPLEX	Acute Gingivostomatitis	Throat Washing	1 - 5	Eggs	Chorioallantoic membrane	Serum, acute and convalescent	Complement Fixation Virus neutralisation
	Herpes labialis	Vesicle fluid	1 - 4				
	Excema herpeticum	Crusts					
	Kerato-conjunctivitis	Conjunctival Scrapings				Herpetic meningo-encephalitis can sometimes be ruled out by examination of a single blood specimen collected before the 5th day.	
	Meningo-encephalitis	C.S.F. Brain (P.M.)	1 - 5				
VARIOLA VACCINIA COWPOX	Smallpox Generalised Vaccinia Accidental vaccination Cowpox	Vesicle fluid Crusts or seeds	-	Eggs	Chorioallantoic membrane	Vesicle Fluid Crusts or Seeds Serum, acute and convalescent	Complement Fixation, for Viral Antigen Complement Fixation
TRACHOMA INCLUSION BLENNORRHOEA	Follicular Conjunctivitis	Conjunctival Scrapings	-	Microscopy for cytoplasmic inclusion bodies			
LYMPHO-GRANULOMA	Lymphogranuloma Inguinale	Pus from bubo	6 - 20	Eggs	Yolk Sac Also microscopy	Serum, acute and convalescent	Complement Fixation

*Not available at present.

Where possible, material for both virus isolation and serology should be submitted. The material for isolation should be stored in the cold compartment of a refrigerator or preferably in a deep freeze and transported to a virus laboratory in ice or CO_2 snow in a thermos flask. In general, serological methods give the most reliable results and are simpler to perform. Two blood specimens (acute and convalescent), however, are necessary for comparison of antibody levels. Rarely can a diagnosis be made on a single blood specimen. Acute specimens should be collected if possible before the 5th day, and the convalescent specimen after the 14th day from the onset of symptoms.

VIRUS DISEASES FOR WHICH THERE ARE NO LABORATORY TESTS AVAILABLE, OR FOR WHICH TESTS ARE TOO DIFFICULT FOR ROUTINE WORK

Infective Hepatitis.
Homologous Serum Jaundice.
Measles.
Rubella (German measles).
Varicella.
Infectious Mononucleosis (other than Paul Bunnell).
Herpes Zoster.
Common Cold.
Viral Gastroenteritis.
Infectious Polyneuritis (Guillain-Barre Syndrome).

INFESTATION WITH THE CHINESE LIVER FLUKE

J. P. WALSH

(*Pathology Department, Green Lane Hospital*)

The following account of a case of clonorchiasis may prove of interest to readers. It is the only case we have encountered at this hospital in the past four years.

Clonorchiasis is a chronic disease caused by the trematode Clonorchis sinensis. It is a member of the fluke family and in the human subject takes up its abode as an adult worm in the bile passages. Here it produces characteristic eggs which migrate to the intestine and are voided in the faeces.

Four years ago a thirty-year-old Chinese seaman was admitted here with a massive left pleural effusion.

In the course of extensive clinical investigation two specimens of faeces were sent to the laboratory for examination for ova and cysts. This examination was prompted following a conversation with the seamen by a sister on the staff who spoke cantonese. She was aware of the incidence of clonorchiasis in China and suggested it be searched for.

The two specimens both revealed "numerous ova of a member of the fluke family, probably Clonorchis sinensis". This was verified by the Parasitology Department at the Central Laboratory.

The eggs were identical with those as seen in the illustration on page 405 of "Clinical Parasitology" by Craig and Faust 1944 edition. A full description of the parasite is given on pages 404 to 409 of that issue and readers are referred to it.

The recovery of the eggs from the faeces was carried out on no less than eight occasions during the patient's eleven months' stay in hospital. Treatment with gentian violet was of no avail.

It is interesting to note that on the three occasions on which differential white blood cell counts were done eosinophils were present only in a percentage of two, three and nine respectively.

The question was raised that this seaman may have acquired the disease on board ship where partially cooked fish possibly infested with encysted cercaria was eaten. However, it seems more likely that his condition was a chronic one acquired in his own country, China.

The technique employed in finding the ova was the time honoured and tested zinc sulphate centrifugal flotation method.

The writer would be interested to learn of the recovery of these eggs on other occasions in New Zealand.

TECHNIQUE FOR CLEANING HAEMATOLOGICAL PIPETTES, ETC.

The technique described below is a modification of the method used in the "Sterile Production Dept." of St. Thomas Hospital, London, developed by James Cole, M.P.S., of St. Thomas Hospital, for cleaning sintered glass filters.

The principle of the method is the destruction of organic matter with an oxidizing agent, acidified 1% potassium permanganate, and the formation of soluble salts by treatment with hydrogen peroxide, which also acts as an oxidizing agent.

REAGENTS

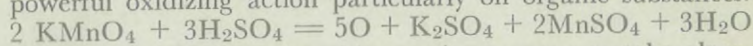
1.
   (a) 10 parts 1% aqueous solution potassium permanganate.
   (b) 1 part concentrated sulphuric acid.

2.
   Acidified hydrogen peroxide. (Made acid with sulphuric acid.)

METHOD

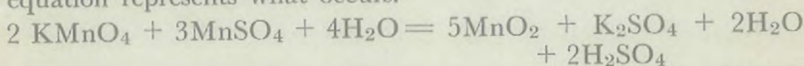
1. Pour freshly prepared solution 1 into container sufficiently high enough to cover upright pipettes.

2. Place pipettes in container in an upright position to allow the acidified potassium permanganate to enter the inside of pipettes. Leave for 30 minutes. During this period nascent oxygen is liberated. The reaction as expressed below has a very powerful oxidizing action particularly on organic substances.



$$2\ KMnO_4 + 3H_2SO_4 = 5O + K_2SO_4 + 2MnSO_4 + 3H_2O$$

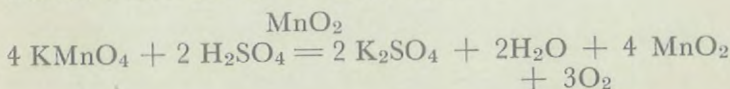
Besides this reaction other side reactions appear to take place. As the sulphuric acid is used up, it is thought that the following equation represents what occurs.



$$2\ KMnO_4 + 3MnSO_4 + 4H_2O = 5MnO_2 + K_2SO_4 + 2H_2O + 2H_2SO_4$$

(Neutral or feebly acid solution) (Acid solution)

The solution now becomes acid again and a further reaction takes place in the presence of the freshly precipitated manganese dioxide which acts as a catalyst thus:—



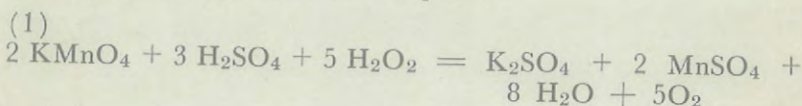
$$4\ KMnO_4 + 2\ H_2SO_4 \overset{MnO_2}{=} 2\ K_2SO_4 + 2H_2O + 4\ MnO_2 + 3O_2$$

Thus a further supply of oxygen is liberated. These side reactions are mentioned because after 30 minutes in the bath of

acidified potassium permanganate, the pipettes appear to be worse than before cleaning began.

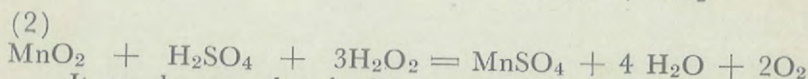
3. With the aid of a vacuum pump and a reservoir, to collect the acidified hydrogen peroxide, suck through the pipette freshly prepared acidified hydrogen peroxide. Also rinse the outside of the pipettes.

The following reactions take place:—



(1)

$$2\ KMnO_4 + 3\ H_2SO_4 + 5\ H_2O_2 = K_2SO_4 + 2\ MnSO_4 + 8\ H_2O + 5O_2$$



(2)

$$MnO_2 + H_2SO_4 + 3H_2O_2 = MnSO_4 + 4\ H_2O + 2O_2$$

It can be seen that further oxygen is liberated and soluble salts are formed. The pipettes are then cleaned and soluble products are washed away by sucking through large quantities of water.

This treatment leaves the pipettes perfectly free from foreign material and spotlessly clean. This method has the added advantage in that its action is not as drastic on glassware as methods which use nitric or chromic acid and it also takes less time.

P.G.S.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

24th and 25th OCTOBER, 1956, at WELLINGTON

Examiners: Dr. D. Allen, Mr G. W. McKinley.

WRITTEN PAPER

Time Allowed: 3 Hours.

1. Discuss the important causes of Blood Transfusion accidents and their prevention so far as this concerns the laboratory.
2. Analyse a method for estimating glucose in blood, indicating principles and sources of error.
3. Answer any two of the following:—
   How would you:
   (a) Calibrate a pipette to deliver 1 ml.
   (b) Prepare an analytical balance for use.
   (c) Prepare an accurate solution of N/10 sodium hydroxide.
4. Describe the origin and development of the leucocytes found in the peripheral blood. Use a diagram to illustrate your answer.
5. Describe the system you would follow in the identification of bacteria.
6. Describe the procedure you would follow in the examination of a specimen of cerebro spinal fluid.

PRACTICAL

Time Allowed: 3 hours Wednesday afternoon and 1 hour Thursday morning for completion of cultures.

1. Do a routine urinalysis on specimens A and B, including cultures. (Albumin, phosphates, casts, R.B.C.s, leucocytes.)
2. You are provided with a solution of potassium permanganate and also N/10 sodium oxalate.
   (a) Estimate the normality of the potassium permanganate solution.
   (b) Showing your calculations only, determine how much water you would add to this potassium permanganate solution to make an exactly N/10 solution.
3. Determine the ABO Group of each of the sera C and D.
4. Draw a sketch diagram of the ruled area of the improved Neubauer counting chamber and give all the linear measurements.
5. (a) Make and fit a cotton wool plug for the tube provided.
   (b) Read the vernier scale provided and record the value.
   (c) Identify the specimen E. (Scolices of E. granulosus.)
6. Identify the culture F. (Shigella schmitz.)
7. Determine the haemoglobin value and do a differential leucocyte count on the blood G.
8. Determine the total non-protein nitrogen of blood specimen H.

ORAL EXAMINATION

Subjects discussed at the Oral Examination were:

Sterilisation, Coombs test and anti-human globulin, icterus index, balances, colorimeters, thermostatic controls, principles of the spectrophotometer, Beer's law, Newton's rings, postal regulations, preparation of N/10 sodium hydroxide, normal and molar solutions, equivalents, milks and waters, albumin in urine, E.S.R.s, C.S.F.s, bile pigments in urine, stercobilinogen, tryptic activity, C. diphtheriae, Sh. shiga, blood films, monocytes, myelocytes, differential counts, micro-pipetting techniques, verniers, false positives for albumin in urine, reducing substances in urine, use of filters in photo-electric apparatus, pH meters, techniques for culturing meningococcus

and Pfeiffer's bacillus, preparation of culture media, testing of organisms for antibiotic sensitivity, reticulocyte and platelet counts.

Of the seven candidates who presented themselves for examination the following were successful:

Mr R. DIX (Blenheim).
Mr T. E. TANNER (Christchurch).
Miss Y. R. YOUNG (Hastings).
Miss J. L. STEVENSON (Auckland).
Miss I. JARMOLICZ (Wellington).
Mr I. LYON (Wellington).

EXAMINATION FOR CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

SEPTEMBER, 1956

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Dr. T. H. Pullar, Dr. S. E. Williams

THEORETICAL PAPER

Sept. 25th, 1956, 9.30 a.m.-12.30 p.m.

1. What findings would you expect in a specimen of C.S.F. from a case of tuberculous meningitis?
   Give a concise outline of the techniques which you would employ in examining such a specimen.
2. What is the commonest cause of erythroblastosis foetalis?
   Discuss the laboratory tests required in the management of a case before and after the birth of the infant.
3. Describe how you would isolate and identify Salmonella typhi from a symptomless carrier of the organism.
4. Write a *short* note on each of the following:—
   (a) Serum amylase.
   (b) Casoni test.
   (c) Decalcification for section-cutting.
   (d) Gamma globulin.

PRACTICAL EXAMINATION I

Tuesday, September 25th, 1956, 2.30 p.m.-5.30 p.m.

1. Determine the absolute reticulocyte count in the specimen of blood provided.
2. Perform a qualitative osmotic fragility test on the blood provided.
3. Stain the given slide by van Gieson's method.
4. Determine the suitability of blood A for transfusion to patient whose serum B is provided.
5. Report on the four blood films provided.
   (Pernicious anaemia, erythroblastosis, malaria (B.T.), hypochromic anaemia.)
6. Identify culture A.
   (S. typhi murium.)
7. Examine throat swab B for pathogenic organisms.
   (C. diphtheriae, H. streptococci.)

N.B.: Questions 6 and 7 are to be completed in Practical sessions on Wednesday and Thursday.

PRACTICAL EXAMINATION II

Wednesday, Sept. 26th, 1956, 9.30 a.m.-12.30 p.m.

1. Estimate the CO_2 combining power of the plasma A. Express in mEqs. per litre.
2. Determine the level of alkaline phosphatase in serum B by the King Armstrong Method.
3. Continue Bacteriology question 6 from Paper I.

PRACTICAL EXAMINATION III

Wednesday, Sept. 26th, 1956, 2.30 p.m.-5.30 p.m.

1. Determine the serum amylase in specimen C.
2. Test the given urine for bile pigments.
3. Continue question 7 from Paper I.

Thursday, Sept. 27th, 1956, 9.30 a.m.-12.30 p.m.

Complete Bacteriology.
Oral.

ORAL EXAMINATION

Subjects discussed at the Oral Examination were:

Types of haemoglobins, estimation of calcium and phosphorus, frog pregnancy test, di-sodium phenyl phosphate, reticulocyte counts, salmonellae, organisms causing gastro-enteritis in children, preparation of Coombs reagent, Bence-Jones protein, differentiation of glucose and lactose in urine, importance of D^u antigen, types of meningitis, C.S.F., sugar levels in meningitis, C.S.F. sugar levels in meningitis, streptococcal infections and typing, identification of pathogenic staphylococcus in blood culture, flame photometry, platelet counts, Feulgen's reaction, Cooley's anaemia, ketosteroids, origin of cells in blood, anaerobic bacilli, differentiation of strains of Myco. tuberculosis, Wassermann reactions and false positives, pneumococci, chlorides in C.S.F., Coombs test in cross-matching, Paul Bunnell test, C. diphtheriae, Addis-Fishberg urine concentration test, abnormal haemoglobin forms in various haemolytic anaemias, acetone in urine with reference to false positives, carbon dioxide values in diabetes, ketone bodies in blood.

Of the nine candidates who presented themselves for examination the following were successful:—

Mr C. K. CLAPSON (Hamilton)
Miss S. A. COOK (Auckland)
Mr D. H. DIGGLE (Westport)
Miss J. A. HELLYER (Napier)
Miss D. E. HITCHCOCK (Wellington)
Mr C. R. HORN (Auckland)
Mr N. D. JOHNSTON (Hamilton)
Mr B. McLEAN (Napier)

BOOK REVIEWS

CLINICAL BIOCHEMISTRY

Abraham Cantarow, M.D.,
&
Max Trumper, Ph.D.,
5th Edition—738 pages—1955. Price 63/-.
Publishers: W. B. Saunders Co., Philadelphia & London.

This is not a textbook of laboratory methods but a further edition of a well-known and highly respected work on clinical biochemistry.

From the time twenty-five years ago when the first edition appeared, the stated aim of the authors has been to bridge the gap, which, though perhaps smaller today, is still present between the literature of so-called "fundamental" biochemistry and physiology and that of clinical medicine. It is further intended to indicate the manner in which the physician may best avail himself of information which can be obtained from biochemical studies. In the application of biochemistry to clinical medicine and surgery, we should be as well acquainted with the limitations as with the significance of biochemical findings in any given case. This volume constitutes an attempt to supply this information and does so in a truly masterly fashion.

The text of the present edition has been virtually completely rewritten. Every chapter has been revised and much new material added, particularly on the following topics: liver function, kidney function, plasma protein abnormalities, biological significance of nucleic acids, uric acid metabolism, porphyrin metabolism, biochemical aspects of diet, iodine metabolism, lipoproteins, fatty liver, potassium metabolism, acid-base balance, endocrine functional diagnosis, especially thyroid and adrenal function.

Carbohydrate metabolism is the first subject and is exhaustively handled in more than seventy pages.

Digestion, absorption and utilisation of glucose, role of the liver and endocrine influences in carbohydrate metabolism are covered.

Abnormalities of post-absorptive blood sugar level, including hyper and hypoglycaemia, abnormal alimentary response and diabetes mellitus are fully described.

Lipid metabolism is thoroughly covered. Protein metabolism is treated well and in an interesting manner, as is the metabolism of nucleic acid, a subject on which readily available information in a handy form is not easily found.

Metabolism of haemoglobin and porphyrins is clearly and concisely described, and here again a clear outline as is given should be valuable as basic information to medical laboratory workers.

The biochemical aspects of diet form an interesting chapter.

The role of various elements including calcium, phosphorus, iodine, sulphur, sodium, potassium and chloride is discussed at length. Water balance is given a separate chapter and includes useful diagrams which should in conjunction with the material presented on "acid-base" (anion-cation) regulation prove valuable in promoting understanding of this important subject.

Respiratory exchange and basal metabolism are dealt with fully, including control of respiration, transport of oxygen, transport of carbon dioxide and anoxaemia. Basal metabolism, including physiological variations on B.M.R. and the clinical significance of B.M.R. are discussed.

The chapter on hormone assay and endocrine function is thorough and up to date.

Finally, hepatic function and renal function are discussed in two excellent chapters. The volume is completed by a description of the chemical constituents of cerebro-spinal fluid.

References to relevant useful literature are provided at the end of each chapter and cross references to related subjects appear throughout.

A comprehensive and well ordered index is provided.

This book is not only a mine of information but is "readable" and should be on the shelves of all laboratories where biochemistry is practised.

F.L.N.C.

MANUAL OF CLINICAL MYCOLOGY

By

N. F. Conant, Ph.D., D. T. Smith, M.D., R. D. Baker, M.D.,
J. L. Callaway, M.D., & D. S. Martin, M.D.

2nd Edition — Published by W. B. Saunders Co.

Price 45/6

The 2nd Edition of the Manual of Clinical Mycology is a book in which the fundamentals of mycology are described intelligibly. The clinical descriptions of diseases caused by mycoses, their differential diagnoses, their pathology and immunology are clearly and concisely written. The laboratory procedures, including techniques for direct and cultural examination of specimens and descriptions for recognition of mycologic cultures are adequate yet simple and easily understood.

The chapters on Actinomycosis, Cryptococcosis, Candidiasis and the three chapters covering symptomatology, prognosis, treatment, immunology and mycology of the Dermatomycoses are of value to N.Z. laboratories.

The appendix is very valuable in that it describes modern mycologic and pathologic methods and techniques. The bibiography at the end of each chapter offers a wealth of sound material. G.R.

DIAGNOSIS AND TREATMENT OF HAEMOPHILIA AND ITS RELATED CONDITIONS

R. G. Macfarlane, Rosemary Biggs

Medical Research Council, Memorandum 32

This memorandum written on behalf of the Committee of the M.R.C. by the above authors gives a description of the distinctive features of various clotting disorders and the modern methods employed in their diagnosis and treatment. Differentiation of haemophilia from other related conditions is relatively easy by laboratory tests. Preliminary tests will include platelet count, bleeding time and whole-blood coagulation time. After these the problem is one of distinguishing between the different types of coagulation defect, and these may be divided into two groups, those in which the one-stage prothrombin time is abnormal such as fibropenia, prothrombin deficiency, Factor V deficiency and Factor VII deficiency; and those in which the one-stage prothrombin time is normal as haemophilia, Christmas disease, and cases with circulating anti-coagulants.

The technical section is divided into four parts; which gives details for the preliminary tests; tests on samples showing a prolonged one-stage prothrombin time which include the two-stage prothrombin test and qualitative tests for Factor V deficiency and Factor VII deficiency; and tests on samples with a normal one-stage prothrombin time which includes the thromboplastin-generation test. The preparation for all special reagents is also given in detail.

The final chapter deals with the general treatment of these coagulation defects. L.E.

ABSTRACTS

STUDIES ON THE EPIDEMIOLOGY OF POLIOMYELITIS IN NEW ZEALAND II

A. M. Murphy

N.Z. med. J. 55, 278, 1956

A report of the results of tests carried out on one hundred and forty-four sera has shown that sixty per cent. of sera had Type I antibodies, fifty-eight per cent. had Type II antibodies and forty-four per cent. had Type III antibodies. Type III antibodies showed a morbid rise in the 15-25 year age group, which the author suggests, shows that the 1937 epidemic was due to Type III virus. The epidemiology of poliomyelitis in New Zealand is shown to be similar to that of the southern part of Australia.

COMPARISON OF TWO COMMONLY USED "SALT-FRACTIONATION" METHODS FOR DIFFERENT PROTEIN ESTIMATIONS

J. K. Fawcett & Victor Wynn

J. clin. Path. 9, 71, 1956

The necessity to verify a method which would give comparable results with those obtained by electrophoretic procedure has caused the authors to compare two common methods for the estimation of plasma protein. The methods tested, Howe's, using 22% sodium sulphate and Majoor's, using 26% sodium sulphate were tested on a series of normal and pathological specimens. Results indicate that although there is little advantage in one method over the other, the method of Majoor may agree more closely with results obtained by electrophoresis.

BACTEROIDES INFECTION

N. P. Markam & C. Kershaw

N.Z. med. J. 55, 308, 1956

The authors report two cases from which members of the species Bacteroides were isolated—B. funduliformis and B. fragalis. They emphasise the necessity of making a special search for these organisms when dealing with pus which is foul smelling or with cases of a septic nature where aerobes are not found. A thioglycollate broth medium is recommended.

A SIMPLE IMPROVED METHOD FOR THE DETERMINATION OF SERUM IRON

Peters et al.

J. Lab. & clin. Med. 48, 280, 1956

An improved method based on the Barkan and Walker procedure, is described for the determination of serum iron. Two millilitres of serum or plasma are treated with 3 ml. of 0.2 N HCl and a drop of thioglycollic acid at room temperature, and then the proteins are precipitated by trichloracetic acid. The iron in an aliquot of the protein-free supernatant is determined by addition of sodium acetate and an alcoholic solution of betho-phenanthroline, which is a reagent twice as sensitive for iron as compounds in general use.

This method has been shown to give consistently complete extraction of iron from fresh or frozen serum specimens, in contrast to some earlier methods. Precision is about $\pm$ 1 mcg. per 100 ml. It does not involve heating, extraction with organic solvents, daily preparation of reagents, or multiple centrifugations. It is applicable to serum or plasma, including cloudy and jaundiced specimens.

A NEW METHOD FOR THE DETERMINATION OF SERUM IRON BINDING CAPACITY

Peters et al.

J. Lab. & clin. Med. **48**, 274, 1956

A simple chemical procedure for the determination of serum iron-binding capacity is described. It requires a sample of only 1 ml. and is applicable to fresh or stored sera and to lipemic or icteric specimens.

The serum is saturated by adding an excess of iron as ferric ammonium citrate. The iron which is in excess of the serum-binding capacity is then removed by addition of a small amount of an anion exchange resin to the centrifuge tube. The serum is diluted and an aliquot of the supernatant analysed for iron content, which is a measure of the total iron-binding capacity of the sample.

Data are presented demonstrating the efficiency of the resin treatment, as shown by studies with Fe^{59}. Values are compared with those obtained on the same specimens by another method, and iron-binding capacity measurements on a series of normal and pathologic sera are given.

SERUM PROTEIN FRACTIONS
A COMPARISON OF PRECIPITATION METHODS WITH ELECTROPHORESIS

B. Levin et al.

J. clin. Path. **3**, 260, 1950

A comparison of the results obtained by using seven different precipitation methods for estimation of true albumin and of gamma globulin and the results obtained by electrophoresis, is presented. The selected methods for a number of normal and pathological sera gave results in good agreement with those obtained by electrophoresis.

QUANTITATIVE ELECTROPHORESIS OF SERUM PROTEINS ON PAPER

Wurm and Epstein

Clin. Chem. **2**, 303, 1956

The authors present a method for paper electrophoresis which they claim gives highly reproducible protein patterns with good resolution and freedom from distortion. They propose a logarithmic relation for the estimation of protein which would seem to apply to any method in use, thus avoiding calculations involving Beer's Law, the validity of which they say should be tested under the particular experimental conditions used. The method is clearly set out and should give valuable technical hints to anyone initiating electrophoretic methods.

HAEMOPHILIA AND ALLIED DISORDERS OF BLOOD COAGULATION

W. R. Pitney, M.D., M.R.A.C.P.; J. V. Dacie, M.D., M.R.C.P.

British Medical Bulletin, 2: 1, 11, 1955

Until recently haemophilia was considered a single entity, the disease diagnosed by clinical history and aided by simple laboratory tests. The abnormal laboratory findings which were considered pathogenic of the disease being

(1) prolonged whole blood coagulation time
(2) diminished consumption of prothrombin

and the disease was shown to be due to a deficiency of the anti-haemophilic globulin (AHG).

Recent advances in knowledge have now shown that a previously unrecognised clotting factor, also seemingly necessary for the production of plasma thromboplastin has recently been identified named the plasma thromboplastin component (PTC) sometimes referred to as the Christmas factor and the disease being called Christmas disease. A third possible type of haemophilia is being considered too, this being caused by a deficiency of a third plasma thromboplastin component named the plasma thromboplastin antecedent (PTA). These three factors all appear to be intimately concerned with the production of plasma thromboplastin, resulting in haemorrhagic states caused by any one of these factors.

Haemophilia is a disease due to inheritance, in true haemophilia AHG deficiency is of sex-linked character transmitted to males through healthy females. Christmas disease is shown to be sex-linked in a similar manner but there may be a difference between the expression of the genes, for mildly affected females have been frequently encountered. PTA deficiency as far as has been studied suggests the defect is transmitted to either sex by an ausomal dominant gene.

The separation of thromboplastin-precursor deficiency states from other forms of haemorrhagic disorders by laboratory findings is relatively easy. Further differentiations between true haemophilia, Christmas disease and PTA deficiency may be made by means of mixing tests, the principle being that bloods deficient in the same constituent will not have their deficiency corrected by mixing, whilst bloods deficient in different factors will mutually correct with each others defect, and more specifically by the thromboplastin-generation test which is the most sensitive of available tests.

For the treatment of haemorrhage in these cases the aim is to correct the defect in the coagulation mechanism, fresh whole blood or fresh plasma contains AHG, PTC and PTA. AHG is unstable in stored plasma, 50% of the potency may be lost during storage for 10 days at 4°C. but the other two factors are more stable on storage.

L.E.

NOTICES

Since this Journal is regularly abstracted by the British Abstracts of Medical Sciences the Editor wishes to draw our attention to a change of title and coverage. The new title is to be:

International Abstracts of Biological Sciences

This decision results from two major developments. The first is an extension of coverage to a wider range of biological research subjects published in journals throughout the world, and the second is that the International Abstracts of Biological Sciences, with the co-operation of the specialist editors selected by the Institute of Scientific Information of the Academy of Sciences of the U.S.S.R. will include, as from the January 1957 issue, translations of the important Russian papers abstracted in the Referativny Zhurnal Biologii (Soviet Biological Abstracts) and Referativny Zhurnal Biologischekoi Khimii (Soviet Abstracts of Biological Chemistry), to be published simultaneously with their appearance in Russian.

International Abstracts of Biological Sciences abstracts the world literature in the following fields: Anatomy, Animal Behaviour, Biochemistry, Biophysics, Cytology, Embryology, Endocrinology, Epidemiology, Experimental Biology, Genetics, Haematology, Histochemistry, Histology, Immunology, Microbiology, Nutrition, Odontology, Parasitology, Pathology, Pharmacology, Physical Anthropology, Physiology, Radiation Effects, Toxicology and Viruses.

EMPLOYMENT

An enquiry has been received from Great Britain from a Bacteriologist qualified as a Fellow of the Institute of Medical Laboratory Technology holding final examinations in Biochemistry and Bacteriology, who desires a position in New Zealand, preferably in Biochemistry. She would be available to start in August. Further enquiries to the Editor.

JUNIOR ESSAY COMPETITION

Entries for both the Technical Study and for the Essay Sections of this competition close with the Editor on June 30, 1957. Entrants must state for which section they wish to enter and on a separate sheet of paper, give their name and address. A prize of £5/5/- is offered for the best entry in each section if it is of a reasonable standard.

SUBSCRIPTIONS

Members are reminded that subscriptions to the Association for the year ending 31st March, 1958, are payable now to the Treasurer:

Mr J. P. Walsh,
Hon. Treasurer,
The N.Z. Association of Bacteriologists,
Green Lane Hospital,
AUCKLAND

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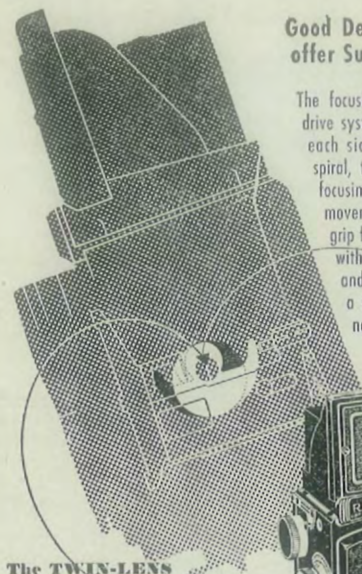

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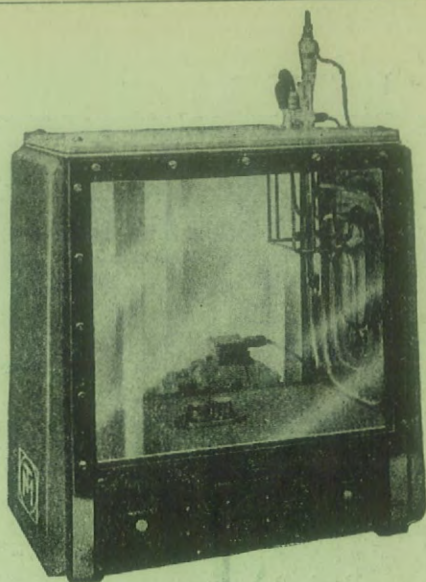

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