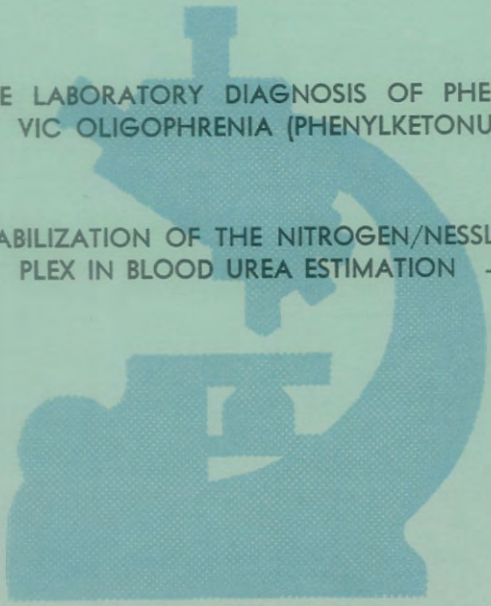
Vol. 16, No. 1 APRIL, 1962

JOURNAL

OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

CONTENTS



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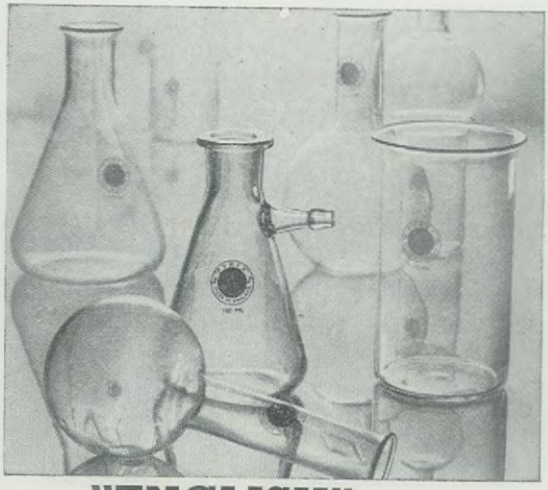

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JOURNAL OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

Vol. 16, No. 1 APRIL, 1962

Communications regarding this JOURNAL should be sent to the Editor, Department of Pathology, Christchurch Public Hospital, Christchurch.

Communications primarily affecting the Institute should be addressed to the Secretary, Mr H. E. Hutchings, Pathology Department, Palmerston North Hospital.

All moneys should be paid to the Treasurer of the New Zealand Institute of Medical Laboratory Technology, Mr D. J. Philip, Pathology Department, Middlemore Hospital, Auckland.

Subscription to this JOURNAL is five shillings per year or two shillings per copy, post free.

Contributions to this JOURNAL are the opinions of the contributor and do not necessarily reflect the policy of the Institute.

ADDRESSES

If the address as printed on this envelope is incorrect, please notify the Editor as soon as possible of your correct address.

THE LABORATORY DIAGNOSIS OF PHENYLPYRUVIC OLIGOPHRENIA (PHENYLKETONURIA)

T. E. Miller

(*Chemical Pathology Department, Public Hospital, Auckland.*)

Phenylketonuria is a hereditary disease characterised by severe mental deficiency and a metabolic defect in the metabolism of phenylalanine. In normal metabolism phenylalanine is hydroxylated to the para and ortho-hydroxy phenylalanine derivatives, with the para hydroxy-phenylalanine pathway being favoured. A small amount of phenylalanine is also metabolised to phenylpyruvic and phenylacetic acid. The phenylketonuric lacks an enzyme for the para hydroxylation of phenylalanine and as a consequence phenylalanine and its metabolites accumulate in the body. The metabolism of phenylalanine is summarized in Fig. I.

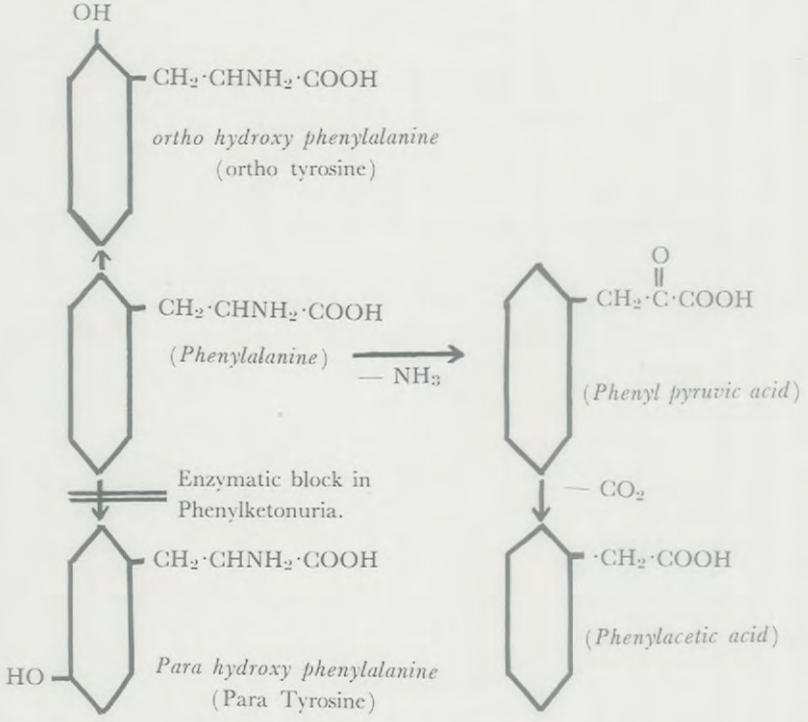


FIG. I. The metabolism of phenylalanine in the body.

In terms of diagnostic laboratory procedures an investigation of this disease should proceed along these lines.

(1) The demonstration of phenylacetic acid in urine.

We have found that a very sensitive test is to shake the urine and smell it. Phenylacetic acid has a characteristic smell rather difficult to describe but suggestive of caramel.

(2) The demonstration of excess amounts of phenylpruvic acid in the urine.

(a) Ferric chloride test. Add 10% aqueous ferric chloride drop by drop to 5 mls. of fresh urine and observe for the production of a green colour. This test can best be performed by adding 10 drops of $FeCl_3$ reagent dropwise to the urine with constant shaking. There should be an interval of about four seconds between the addition of each drop. There are two practical points in the performance of this simple test. The first is that the urine should be slightly acid and the second is the necessity for the rapid removal of ferric phosphate if any should form either by centrifuging or filtration. The green colour fades in about five minutes and could be masked by the presence of a heavy precipitate of ferric phosphate.

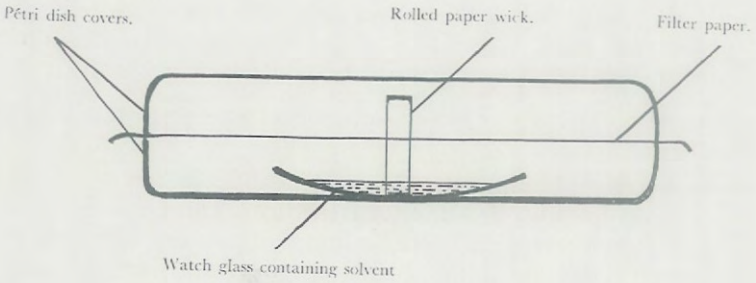
(b) The reaction with 2, 4, dinitrophenylhydrazine.

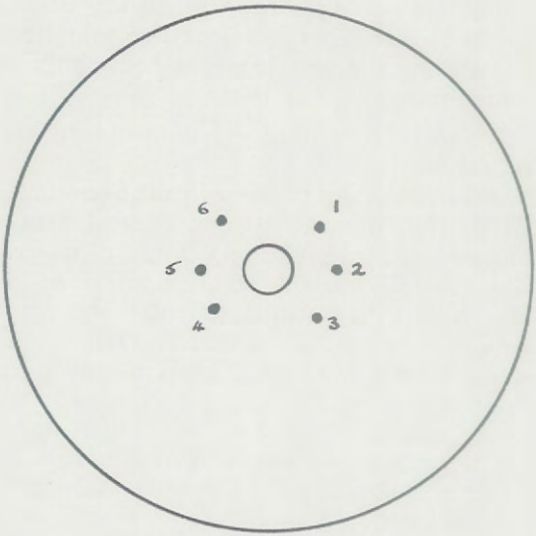
Reagent.

Stock 2, 4, dinitrophenylhydrazine—0.2% in normal HCl. For use add 10 mls. of stock reagent to 10 mls. of concentrated HCl and 80 mls. of water. To perform the test mix one ml. of urine and one ml. of the 2, 4, dinitrophenylhydrazine reagent. Stand for 30 minutes and add 2 mls. of normal NaOH. A normal control urine should be tested simultaneously. A positive test is indicated by the appearance of a cherry red colour while normal urines show only a light brown colour with this reagent. The test however is not specific for phenylpyruvic acid and gives a positive reaction with aldehydes and ketones.

(3) The demonstration of increased amounts of phenylalanine in the urine.

To demonstrate this we used a radial paper chromatograph. The apparatus (Fig. II) consists of the lids of two 6-inch Petri dishes, a watch glass capable of holding 16 mls. of fluid, a rolled paper wick and a piece of Whatman No. 1 circular filter paper (18.5cm. diameter) which has a hole 8 mm. in diameter bored neatly through the centre. The filter paper is sandwiched between the two Petri dishes.





WHATMAN'S NO. 1 FILTER PAPER (18·5 CM DIAMETER)
WITH STARTING POINTS MARKED.

FIG. II. Apparatus for the demonstration of phenylalanine in urine by radial paper chromatography.

The paper is prepared by first describing a circle of radius 2.0 cm. from the centre of the hole and then marking up to six equidistant spots around the circumference of the circle. This operation should be carried out using a soft lead pencil and light markings. The urinary nitrogen content of the specimen is then estimated and a total of 50 μg of nitrogen spotted onto the paper (usually about 10 μl). This is best accomplished by the use of a haemoglobin pipette. A small volume of urine, the diameter of which should not exceed 5 mm. is applied to the spot and then dried in a current of warm air. A further aliquot is then superimposed on this spot and the procedure repeated until all the sample is transferred onto the paper. A standard 2 μg spot of phenylalanine and a normal control urine should be run with any urine under investigation. A wick is then made by cutting a strip of Whatman No. 1 paper 15 cm. x 2 cm. and formed by winding around a thin glass rod. The wick is then firmly fitted into the hole bored in the filter paper. The solvent, N butanol, ethanol, water, 7 ml., 2 ml., 2ml., is prepared and poured onto the watch glass. The chromatogram with wick attached is then placed in position and the cover lid applied. The wick should dip into the solvent and support the centre of the paper. Solvent will flow up the wick and radiate towards the periphery of the paper. When the solvent has reached the Petri dish covers the paper is removed, the wick discarded, solvent front marked and the paper dried in a current of warm air. 0.1% ninhydrin in normal butanol is then sprayed or brushed onto the paper which is heated at 80°C for 30 minutes. With this solvent common urinary amino acids apart from phenylalanine do not move readily. Thus a very informative chromatograph as seen in Figure III may be obtained.

In all manipulations it is important not to touch the filter paper with the fingers as finger prints stain with ninhydrin solution. When spotting on the urine fold a further piece of filter paper over the chromatogram and grip this.

(4) The demonstration of increased amounts of phenylalanine in the serum.

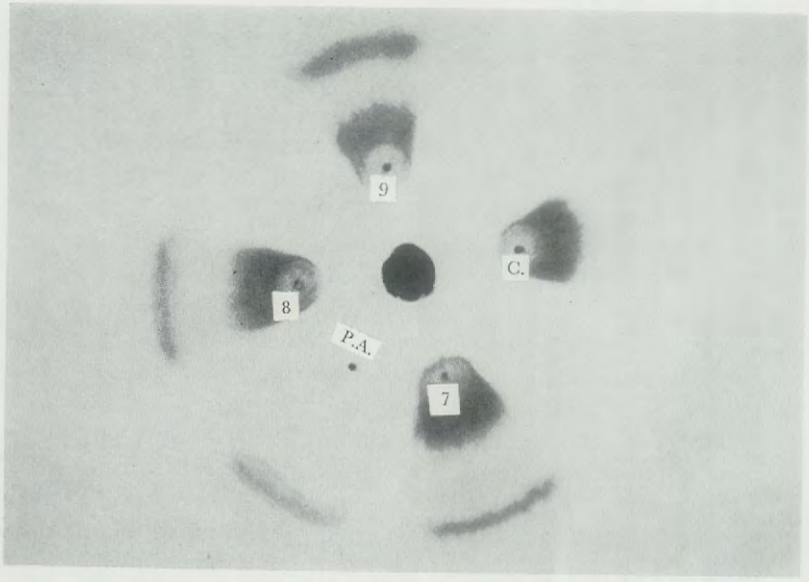


FIG. III. Radial chromatograph of urine from a phenylketonuric patient showing the band of phenylalanine.
7, 8, 9 = urine specimens taken on successive days.
P.A. = a standard 2 μg phenylalanine spot.
C = a normal control urine.

The normal level of phenylalanine in the serum is 1-2 mg./100 ml. Howeyer in phenylketonurics the level may be as high as 60 mg./100ml. To demonstrate any increase we use a paper chromatographic technique (Berry, 1957). Although quantitative estimation of serum phenylalanine by this method is subject to fairly large errors it is quite adequate as a diagnostic aid.

DISCUSSION:

There is an increasing interest in the diagnosis of phenylketonuria in this country. A programme directed by the Department of Health is now under way whereby parents are supplied with a test paper and asked to test their infants' urine with this paper when the child is three weeks of age. The Plunket Society also test the urine of infants visited at their homes with ferric chloride reagent. These tests are performed at the age of two, four and six weeks. At the present birth rate in New Zealand we would expect two or three of the infants born during the year to be phenylketonurics.

An early diagnosis is desirable as affected infants can be helped by maintaining them on a low phenylalanine diet.

As the disease is a rare one most laboratories have probably not had a great deal of experience with laboratory procedures that may be used as a diagnostic aid. The techniques described in this paper are simple, give valuable results and are within the scope of the average laboratory.

SUMMARY:

The metabolism of phenylalanine in phenylketonuria is discussed and four procedures for the laboratory diagnosis of this disease described.

ACKNOWLEDGEMENTS:

The author is indebted to Dr. R. O. Farrelly for his direction during the course of this work and to Miss Ruth Carr for her illustrations.

REFERENCE:

Berry (1957). Proc. Soc. Exptl. Biol. Med., **95**, 71.

STABILIZATION OF THE NITROGEN/NESSLER COMPLEX IN BLOOD UREA ESTIMATIONS

A. J. Forsyth

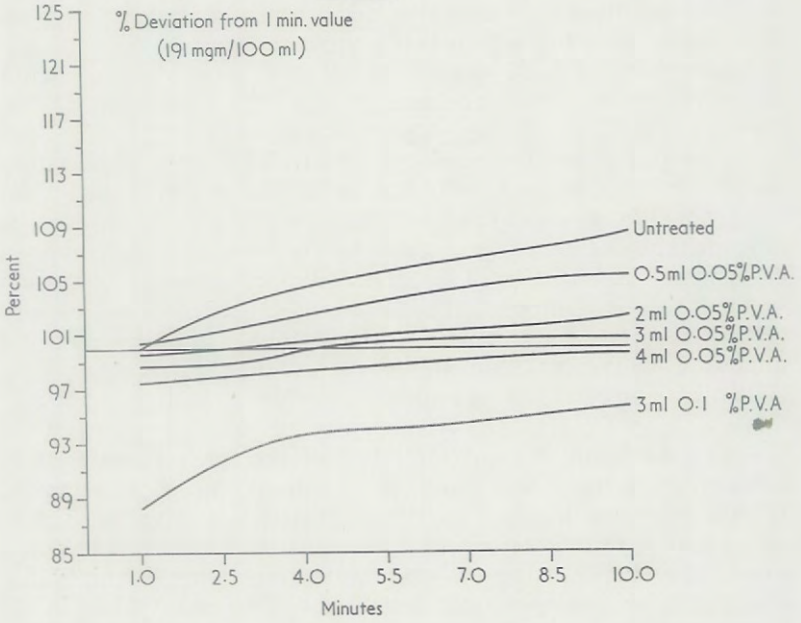
(*Chemical Pathology Dept., Medical School, Dunedin*)

It has been observed for some time that an aqueous solution of ammonia, after Nesslerization, grows increasingly turbid, and after three or four hours will flocculate. These investigations have been made on the turbidity in the first 10 minutes, and on the attempts of using Poly Vinyl Alcohol and Gum Ghatti to hold the Nitrogen/Nessler complex in a true colloidal state. The object was to find a method of stabilizing the optical density, so that urea estimations need not be read immediately after Nesslerization, but could be done at the technologists convenience, and yet still be accurate.

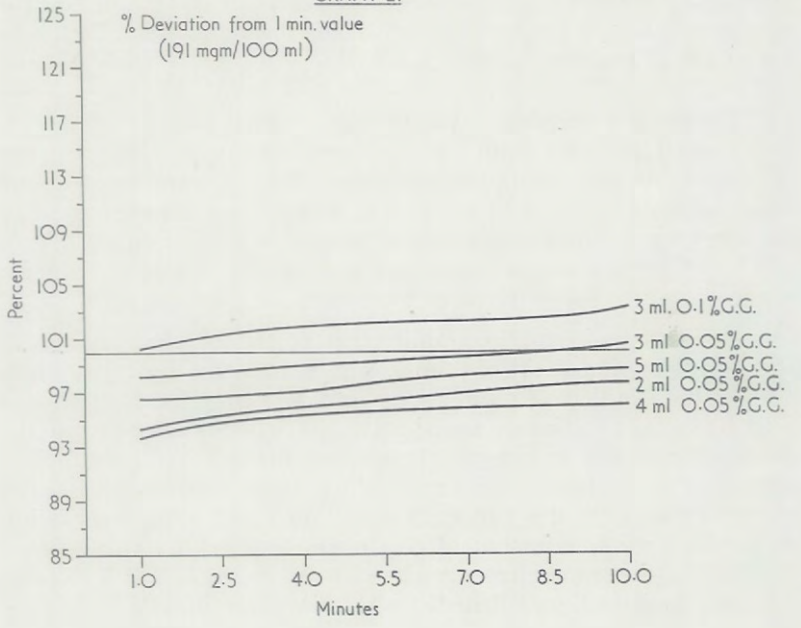
The method used is that set out in Varley (1958). In order to avoid error caused by varying amounts of urease, the enzyme reaction was carried out in bulk, and suitable aliquots processed. One other modification has been made, that is to read each estimation exactly one minute after the addition of the Nessler solution. Using urea solutions of known concentration, this modification has been found to return values always between 98.5% and 100% of the true value. This is a standard procedure in this laboratory, and will be referred to at the conclusion. Bulb pipettes were used for all solutions, and all optical densities read in a late model Spekker colorimeter. All estimations were done in quadruplicate and the mean values taken. In all over 500 readings were made. For purposes of comparison, the value obtained with an untreated test, read exactly one minute after Nesslerization, has been taken as 100%, and all other values have been expressed relative to this. A high and a low urea, of 191 mg./100 ml., and 22 mg./100 ml. respectively were used, both derived from pooled human blood. There is only about 2 mg./100 ml. difference between blood and serum urea levels. It has been found more convenient to put the results in the form of graphs.

Graph 1 shows the effect on the high urea of various volumes of 0.05% Poly Vinyl Alcohol. This was introduced in the final dilution with the 5 ml. of water, the final volume, excluding Nessler, being brought to 7 ml. in each case. An untreated test shows a considerable increase in turbidity, 8.5% after 10 minutes. Addition of even 0.5 ml. of the P.V.A. is enough to reduce the turbidity noticeably. Larger volumes reduce the turbidity even further, but past 4 ml. the P.V.A. appears to be similar to chlorides,

GRAPH 1.



GRAPH 2.



and inhibits the formation of the Nitrogen/Nessler complex, so the one minute values are lowered. 3 ml. of P.V.A. gives the best results, allowing only a 0.1% increase between the 7 and 10 minute values. P.V.A. appears to have no blank value of significance, relative to these high ureas.

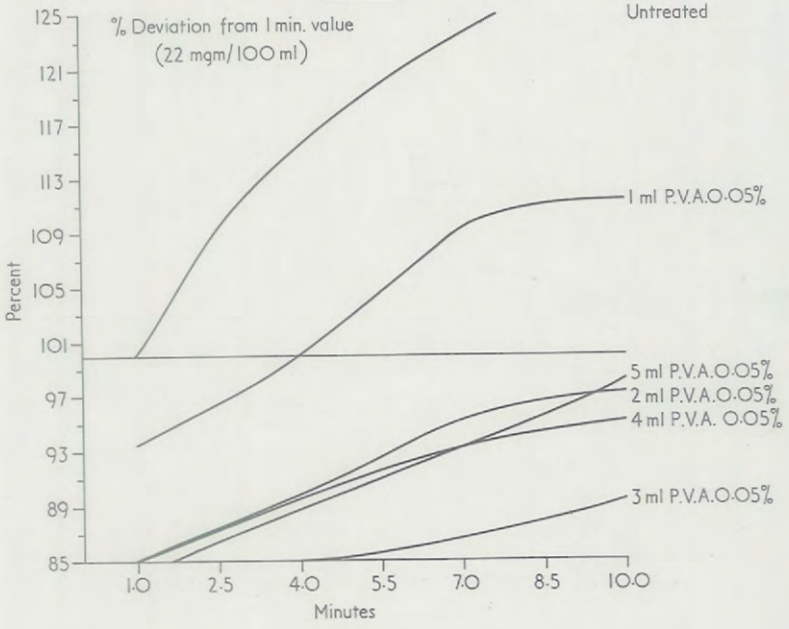
Graph 2 shows the result of substituting 0.05% Gum Ghatti for the P.V.A. Gum Ghatti has an inhibiting effect on the ion formation but it also appears to have a blank value due to the nitrogen content or other interfering chromogens which opposed this effect. 2ml. of Gum Ghatti inhibits the formation of the ion, so the curve is depressed. For 3 ml. the apparent nitrogen content raises it to near the 100% line. For 4 ml. however, the inhibition is greater than the effect of the apparent nitrogen content. The 3 ml. curve appears to be the most satisfactory.

Graphs 3 and 4 show the effect of the same quantities and concentrations of P.V.A. and Gum Ghatti, on the low ureas. With a low urea, the increase due to turbidity is of greater significance, an untreated test rising 30% in 10 minutes. For low urea levels, the P.V.A. appears to exert a variable blank value, giving similar results to that obtained with Gum Ghatti in the previous graph. The 2 ml. curve is the best, although far from satisfactory. For Gum Ghatti in low level ureas (graph 4), the apparent nitrogen content far outweighs the inhibitory effect, and no curves of practical value result.

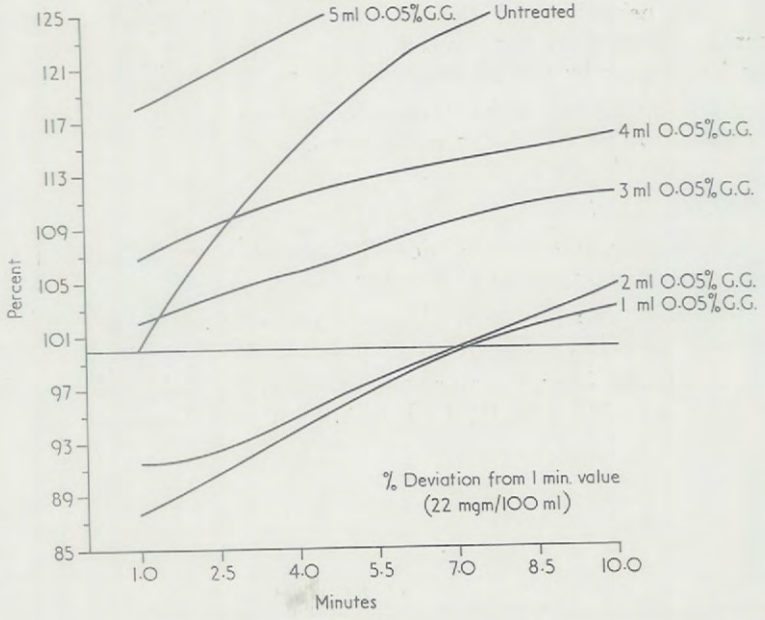
Graph 5 gives the curves for the optimal volume of P.V.A. and Gum Ghatti for both the high and low ureas. The amount of reagent needed to hold the Nitrogen/Nessler complex in a true colloidal state appears to be related to the urea concentration of the specimen. There is apparently no one amount of either P.V.A. of Gum Ghatti which will stabilize the complex ion over the wide range of values encountered in laboratory estimations.

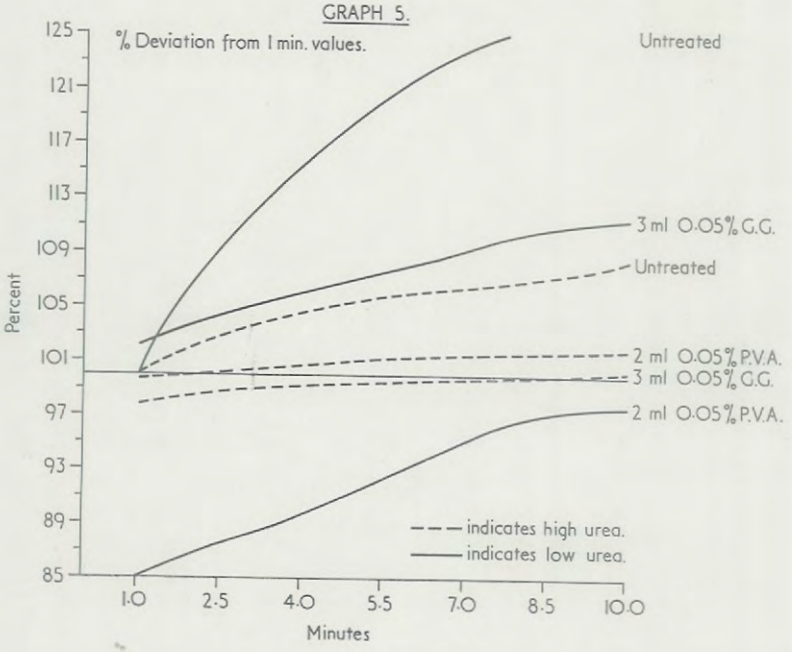
A further aid to the stabilization of the Nessler complex is the addition of a drop on iodine solution to the filtrate prior to adding the Nessler solution (King and Wootton 1956) This was found to effect an initial drop in density but did not prevent the density increasing as before. It appears that the only method of obtaining accurate results is to introduce the modification as set out at the beginning of this paper. Varley's method for the preparation of Nessler's solution is that of Koch and McKeekin (Hawk et. al 1954), but the same curves result with the Folin and Wu, Nessler solutions and Bock and Benedict's (Hawk et. al. 1954).

GRAPH 3.



GRAPH 4.





The behaviour of the Nitrogen/Nessler complex therefore seems unaltered by the concentrations of mercury and iodine in the Nessler, or by the normality of the sodium hydroxide present.

I would like to thank Messrs R. D. Allan and J. V. Dunckley for their help and advice in these investigations.

REFERENCES:

1. Varley, Practical Clinical Biochemistry 2nd Ed., 1958. W. Heinemann Ltd., London.
2. King and Wootton, Microanalysis in Medical Biochemistry 3rd Ed., 1956. J. & A. Churchill Ltd., London.
3. Hawk, Oser, Summerson, Practical Physiological Chemistry 13th Ed., 1954. J. & A. Churchill, London.

CONFERENCE REPORT

Report from the second annual one-day Conference held by the Auckland Branch N.Z.I.M.L.T. at the Medical Centre, Auckland Public Hospital, on Saturday, 4th November, 1961.

The conference was attended by approximately 60 people during the course of the day and 15 visitors came from Kaitaia through to Tauranga.

Mr Meredith, the Branch chairman, welcomed the visitors to the conference and then Dr. S. Williams, the Director of Laboratory Services, gave the opening address.

In the course of his address he complimented the Branch on the organisation of the conference and then spoke in some detail on the proposed new training system that will be starting in the Auckland Hospital in 1962.

After morning tea the papers were given and they included:

Mr T. E. Miller: (1) Some experiences in the identification of urinary amino-acids by paper chromatography; (2) The laboratory diagnosis of phenylketonuria; (3) The effect of an extraneous chromagen on the endogenous creatinine clearance.

Mr A. Fischmann: Auto-immunity and false positive serological findings.

Mr S. Philip: The recovery of a tapeworm.

Mr B. White: Radio-active isotopes.

Mr E. Clarke: Difficulties involved in antigen-antibody reactions.

Mr J. France: Some aspects in Steroid chemistry.

Mr Orbell: An inhibitor that occurs in some batches of nutrient agar.

Mr G. Davies: Quality controls.

Mr J. Holland: Salmonella typing.

Mr W. Wiggle: The study of Potassium levels *in vito* over a three-hour period.

The conference was followed by a cocktail party and dance at the Parnell Rose Gardens.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY (INC.)

COUNCIL MEETING OF OCTOBER 28th, 1961

Present at the meeting: Messrs H. Olive (in the chair), H. Bloore, H. Hutchings, D. Philip, G. Cameron, J. Walker, G. Rose and Miss J. O'Grady.

Moved: That apologies be received from Miss Mattingly and Mr Donnell. Bloore/Rose.

Mr Olive welcomed Miss O'Grady to the council as junior member.

Minutes of previous meeting.

Moved: That the minutes be confirmed. Cameron/Walker.

Applications and Resignations.

Applications from the following were received:

Junior members: Miss R. Hughes, Rotorua; Mr R. E. Clifton, Dunedin; Mr E. M. Johnson, Thames; Miss P. M. Anderson, Wellington; Miss J. Coates, Wellington; Mr K. Bateman, Lower Hutt; Miss J. R. Nicholls, Lower Hutt, Miss A. Ward, Lower Hutt; Miss J. A. Maseyk, Lower Hutt; Miss P. Cox, Auckland; Miss A. G. Lee, Invercargill; Mr B. Ch. Cresswell, Christchurch; Miss J. Garner, Lower Hutt; Miss L. Martin, Lower Hutt; Miss B. O'Reilly, Lower Hutt, Mr W. Haigh, Auckland.

Senior Members: Mr D. Taylor, Miss S. R. Fraser.

Resignations.

Moved: That applications be approved and resignations accepted. Philip/O'Grady.

Treasurer's Report.

The treasurer reported that 47 senior members and 142 junior members were unfinancial.

There was £203/10/11 outgoing and a balance of £238/2/10.

Moved: That the treasurer's report be adopted. Philip/Rose.

Editor's Report.

The Editor reported that the current journal was in the hands of the printer.

There was, he declared, nothing in hand for publication, even from the Conference. The next publication was due in April.

Moved: That the Editor's report be received. Walker/Bloore.

Correspondence.

Moved: That the Christchurch branch be recognised and the rules as submitted be approved. Cameron/Rose.

Correspondence.

From Auckland re application for reconsideration of salaries and could Branches write to the Department concerning such applications.

It was noted that Rule 5B of Branch Rules precluded a Branch from making individual applications.

Submissions on salaries were to be considered at this meeting.

The Secretary was asked to write asking for clarification of Registration of Branches.

Correspondence.

From Dunedin Branch re use of microscopes at examinations.

It was noted that should all recommendations be adopted personal microscopes would no longer need to be taken from the laboratories.

Re reciprocity with U.K. I.M.L.T.

The Secretary was asked to write to the Secretary of I.M.L.T. applying for official recognition of C.O.P. to the extent that the holders may have the right to automatically sit for an associate of I.M.L.T. The Secretary was asked to explain in his letter the local situation and the changes hoped for with a view to full reciprocity in the future.

A letter from Mr Norman of England precipitated the need for this letter where before it had been hoped to establish the changes before applying for complete reciprocity.

Moved: That the Wellington Branch be recognised and the Rules as submitted be approved.

Moved: That inward correspondence be received and outward correspondence be approved. Bloore/Walker.

General Business.

The Secretary was asked to write a letter of sympathy to Mr and Mrs Christensen at the death of their daughter.

Information was tabled on the concessions allowed Radiographers by the N.A.C.

As they appeared more lenient than those indicated to the N.Z. I.M.L.T. the Secretary was asked to write to the N.A.C. for further information.

Regional representation.

As requested by the floor of the Conference the Council gave consideration to Regional Representation.

Moved: That a letter be sent to the Dunedin Branch expressing sympathy for their request but pointing out that it was felt that disadvantages outweighed the advantages at this time. Bloore/Walker.

Discussion was had on the progress the recommendations to the Department of Health on the training scheme for Technologists. The first essential step was recognised as being the formation of an examining Board. The recommendations gave the responsibility of the syllabus to this Board. Until the Minister of Health approved the recommendations, no official moves could be made.

Nominees to Hospital Laboratories Advisory Committee.

The following names were put forwards: Mr Bloore, Mr Reynolds, Mr Whillans, Mr Olive, Mr Hutchings in accordance with Hospital Board Employees (conditions of employment) Regulations 1959 Clause 4 Section 1 subsection B.

Submissions to Salaries Advisory Committee.

After some discussion the following submissions were made:

Special Grade up to £2,300.

Grade A	£1,600 — £1,750	— £1900		
Grade B	£1,400 — £1,500	— £1,600	— £1,700	
Grade C	£1,210 — £1,300	— £1,400	— £1,500	
Grade D	£1,075 — £1,120	— £1,165	— £1,210	— £1,300

That "double time" rates apply for work between midnight and 8 a.m.

Resubmission of:—

That consideration be given to the grading of positions available in hospitals, and that personal grading be additional and separate to that of the position.

That consideration be given to the inclusion of provisions to cover the employment of Tutor Technologists.

Confirm that the council supports the recommendations made by Dr D. T. Stewart on behalf of the Society of Pathologists with reference to the employment of Science graduates.

Moved: That the expenses of the meeting be paid. Olive/O'Grady.

The meeting closed at 4.00 p.m.

REX AITKEN PRIZE

Members of the Institute wishing to enter for this prize should have their entries in the hands of the Editor of this journal by the 31st May, 1962. Entries are to be reprints or copies of articles published during 1961 and be of a technical or practical nature.

JUNIOR ESSAY COMPETITION

Entries for the Technical and Essay Sections close with the Editor of this journal on June 22nd, 1962.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Wednesday, 18th October, 1961.

Examiners: Dr J. O'Brien and Mr A. Harper.

All questions carry equal marks.

WRITTEN PAPER—Three hours.

(1) A. (i) **Briefly** outline your technique for the direct examination for M. tuberculosis in a batch of sputum specimens.
(ii) **List** errors which may result in a false positive report when the batch contains both positive and negative samples.
B. How would you sterilise the following items? **In a few words** state the reason for your choice.
Serum for Loeffler's Medium.
Discarded cultures.
Rubber gloves.
Selenite F enrichment media.
Paraffin oil.

(2) Describe **briefly** a technique for isolating each of the following organisms from heavily contaminated material. State **in a few words** the principle on which your methods depend.
Staphylococci.
S. typhi.
N. gonorrhoeae.
M. tuberculosis.

(3) A. Give an account of the errors which may occur when performing a blood sugar estimation.
B. Write **short** answers to the following:—
(i) How does Benedict's quantitative reagent differ from the qualitative?
(ii) What adjustments may be necessary to a sample of urine prior to carrying out Esbach's test?

(4) Describe your technique for carrying out a reticulocyte count. What is the composition of the reagent used? **Name** the general term used to describe the type of staining used.

(5) A. **Enumerate** the causes of a false negative result in the Direct Coomb's test.
B. **Name** the pre-requisites required in water to be used for Acid Citrate Dextrose.
C. Describe **briefly** the preparation of new microscope slides to be used for blood films.

Wednesday, 18th October, 1961.

PRACTICAL PAPER I—Three hours.

(1) Prepare a blood agar plate with the material provided.
(2) Examine microscopically and culture Urine A. (Coagulase positive Staphylococcus).
(3) Examine routinely the Urine B. Culture not required. (WBC's, RBC's, Casts, Sugar and Albumin).
(4) Examine the swab of Pus C. (β—haemolytic streptococcus).
(5) Examine faeces D. for occult blood.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Thursday, 19th October, 1961.

PRACTICAL PAPER II—Three hours.

(1) Complete the Bacteriology from yesterday.

(2) Make a platelet count on the blood A provided. (Include in your answer your visual count and subsequent calculations.)

(3) Prepare and stain a film from the blood A provided.

(4) Determine the A B O and Rh. group of the blood A provided.

(5) Examine and report on the labelled slides at the separate microscope.

1. Erythroblastosis foetalis.
2. Gonococci.
3. Basophilic stippling.
4. Lymphatic leukaemia.
5. P.A.

ORAL EXAMINATIONS

Leishman stain, Reticulocytes, Hot-air ovens, Thermostats, Glycolysis, Protein precipitants, Urine S.G. Filters, Protein estimations, sensitivity tests.

The following candidates were successful:

Mr R. C. McHardy, Wellington.
Mr R. J. Nicholas, Palmerston North.
Miss M. H. F. Cox, Christchurch.
Miss J. A. Morrison, Balclutha.
Mr B. J. P. Elliott, Auckland.
Mr D. W. Long, Auckland.
Miss P. A. Joy, Auckland.
Miss E. A. Hay, Auckland.
Miss B. L. Bailey, Dunedin.
Miss A. M. Macphail, Blenheim.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

Tuesday, 22nd August, 1961.

Examiners: Dr. J. D. Reid, Dr N. Markham, Mr J. T. Murray.

WRITTEN EXAMINATION

Time allowed, three hours.

Answer ALL Questions

1. (a) Write a critical account of the methods used to isolate **Mycobacterium tuberculosis** from human sources and the methods used for assessing drug sensitivity. State what precautions you should take when handling tuberculous materials.

Make a list of any other Mycobacteria which can cause disease in man and state the type of material from which the organisms may be recovered.

(b) Write brief notes on the following:

1. Craigie Tube.
2. The use of neomycin in culture medium.

3. Routine Test Dose as used in bacteriophage typing.
4. Selective or Enrichment Media.

2. Describe the principles involved and details of the Van Slyke method for the estimation of the CO_2 combining power of serum (alkali reserve). What precautions would you observe to ensure that the results are accurate?

   Give the normal range in volumes % and in m.M. per litre (m.Eq. per litre). What values would you expect in the following conditions?

   (a) Severe diabetic coma.
   (b) Renal failure.
   (c) Pyloric obstruction with prolonged vomiting.
   (d) Severe chronic respiratory disease.

3. What laboratory tests for evaluating renal function do you know? Describe each **briefly,** give the normal values or range for each test and mention any precautions to be taken in carrying out the tests so that the results may be reliable.

4. Discuss abnormalities of red cells as seen in ordinary stained blood films which have diagnostic or suggestive clinical values.

5. (a) Discuss the principles underlying the reactions seen when the following stains are used:—
   (a) Haematoxylin and Eosin.
   (b) Feulgen.
   (c) Leishman.

   (b) Discuss the reliability of the red cell count.

N.B.—Question 1 carries 33 1-3% of the marks for this paper; all others carry 16 2-3% only.

PRACTICAL I

Tuesday, 22nd August, 1961.

2.30-5.30 p.m.

BACTERIOLOGY I

1. A culture (A) of an organism isolated from the C.S.F. of a child aged 2½ years.

   Identify the organism and state what abnormal changes would have occurred in the C.S.F.

2. Culture (B) of a swab from a deep infected wound.

   What is the medium used and what are its special properties?
   Identify the organisms present and outline any additional tests necessary for their complete identification.

3. A swab (C) from an encrusted lesion on the leg of a child.

   Culture and identify the organisms as far as time permits.
   Outline any additional investigations that you think should be made.

4. A culture (D) of an organism isolated from the throat of a patient who had had intensive antibiotic therapy. The tonsils of the patient were covered with a creamy white exudate.

   Identify the organism as far as you can, and give any additional tests which you consider necessary.

HAEMATOLOGY I

1. You are provided with the following sera:
   a. Unadsorbed.
   b. Adsorbed with boiled guinea pig kidney.
   c. Adsorbed with boiled ox red cells.
   Perform a Paul Bunnell test and state your interpretation of results.
2. Perform an osmotic fragility test on the blood provided.

PRACTICAL II

Wednesday, 23rd August, 1961.

9.30 a.m.-12.30 p.m.

BACTERIOLOGY II

1. Complete questions 1-4 from yesterday.
2. Write notes on the "Spots" A to E.
   (a) Blood film (Plasmodium falciparum).
   (b) Faecal concentrate (Ova, Ascaris, Ankylostoma, Trichuris).
   (c) Blood film (Filariae).
   (d) Specimen (Ascaris lumbricoides).
   (e) Slide culture (Microsporum canis).

HAEMATOLOGY II

1. Examine and report on blood films A, B, C and D.
   (a) Glandular Fever.
   (b) Myeloid leukaemia (Chronic).
   (c) Pernicious anaemia.
   (d) Haemolytic anaemia (Spherocytosis).

PRACTICAL III

Wednesday, 28th August, 1961.

2.30-5.30 p.m.

BIOCHEMISTRY (three hours)

Group I

1. You are in charge of a small laboratory and have just received a new spectrophotometer. (The instrument provided.) Calibrate this instrument for the estimation of serum urea **and as part** of this calibration demonstrate whether Beer's Law is followed with this instrument. Show your results both graphically and numerically. Specimen A is a stock nitrogen standard.

   Determine the serum urea level on Specimen B which is a filtrate made after incubating serum with urease.

   Briefly describe the procedure you have carried out and show all calculations.

Group II

2. (a) Specimen C is urine from a diabetic woman who is suspected of aspirin poisoning. Carry out tests to show whether or not this patient has taken an overdose of aspirin.
   (b) Specimen D is urine from a soldier who has just completed a long route march. What does this urine contain? Carry out confirmatory tests. (Centrifugal deposit: Microscopic: Nil of note.)

(c) Specimens E and F are two specimens of blood, one of which is from the cord of a new born infant and the other is adult blood. Show **biochemically** which is cord and which is adult blood. Write a short note on your findings.

(d) Specimen G is serum from a patient who has had a **severe** transfusion reaction 12 to 15 hours previously. Determine as far as you can what pigment or pigments have caused the abnormal colour of the serum.

Note: In the above questions briefly describe what tests you have carried out and the results of those tests.

Group III

3. (a) You pH meter is out of action and you wish to determine the pH of Specimen H which should be pH 7.4. With the equipment and reagents provided determine the pH of this specimen. Describe briefly your method and the result.

(b) Write notes on exhibits Nos. 1 to 5.

1. Calomel Electrode.
2. Two electrophoresis strips.
3. Selenium cell.
4. Ampoule Gold chloride.
5. TNPN with excess acid.

ORAL EXAMINATION

Dr Reid

Reason for the cause of jaundice in a new born baby with a negative direct Coombs, ABO incompatibility, immune anti-A, use and constituents of Ehrlich's reagent, Diazo reagent, have to distinguish between urobilinogen and porphobilinogen, preparation of Coombs reagent, Bilirubin methods for newborn babies, Fisher-Race Terminology, Wiener Terminology.

Dr. Markham

Counting of bacteria, counting of viable bacteria, testing water samples, W.R. reagents and tests for syphilis, autoclaving of theatre material, precautions with use of autoclaves and placing of material, tests for sterility, Salmonella and Shigella.

Mr Murray

Spectroscopic appearances of methaemoglobin and sulphaemoglobin, principle of dark ground condenser, Bilirubin standards, Nessler's reagent, definition of micron, anticoagulants, numerical aperture.

The following candidates were successful:

Mr L. R. Taylor.
Miss G. M. Stairmand.
Mrs J. N. Woodfield.
Mr D. A. McArthur.
Miss G. M. Collyer.
Mr D. J. Dunlop.
Mr C. L. Stenbeck.
Mr N. L. Harris.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

Examiners:

Palmerston North: Dr T. H. Pullar, Mr L. Reynolds, Mr H. E. Hutchings.
Auckland: Dr. S. Williams, Mr H. G. T. Olive, Mr D. Whillans.
Christchurch: Dr N. P. Markham, Mr G. W. McKinley, Mr J. T. Murray

Tuesday, 6th March, 1962.

2.30-5.30 p.m.

WRITTEN PAPER I—HAEMATOLOGY AND BLOOD BANK TECHNIQUE

Time allowed, three hours.

Answer ALL Questions.

1. (a) Give an outline of the **main** factors involved in the coagulation of blood.

   (b) A young man requires to have several carious teeth extracted, but states that he is a "bleeder." Describe briefly the laboratory tests that would be necessary for the full investigation of such a case.

2. State what you know about "acquired haemolytic anaemia," with special reference to the laboratory tests required for confirmation of this diagnosis, and any difficulties that may be associated with blood transfusion in this condition. (20 marks.)

3. Write short notes on:—

   (a) Heinz bodies.

   (b) Mean corpuscular haemoglobin concentration.

   (c) Exchange transfusion in erythroblastosis foetalis.

   (d) Rose-Waaler test. (20 marks.)

4. (a) What are the optimum conditions for the storage of blood in a "Blood Bank"?

   (b) What disadvantages are there (if any) in giving a transfusion of blood a week old, as compared with fresh blood?

   (c) Describe any **one** method for processing plasma from time-expired citrated blood. (30 marks.)

Wednesday, 7th March, 1962.

9.30 a.m.-12.30 p.m.

WRITTEN PAPER—BIOCHEMISTRY

Time allowed, three hours.

Answer ALL Questions.

(Each of the five questions carries 20 marks.)

1. For many biochemical analyses it is necessary to precipitate blood proteins. Describe the various methods of carrying out this process and give reasons for adopting different methods.

2. Discuss briefly:

   (a) Methods of controlling accuracy in a Hospital Biochemistry Laboratory.

   (b) The sources of error in the estimation of non-protein Nitrogen.

3. Discuss the principles involved in spectrophotometry.

   What is Beer's Law?

   Write briefly on the difference between Optical Density and percent Transmission.

4. What methods are available for gasometric analyses in a Hospital Biochemistry Laboratory?

   Write brief notes on the estimations carried out by these methods.

5. Write brief notes on any five of the following:

   (a) Folin & Ciocalteau Reagent.

   (b) Molar solution.

   (c) milliEquivalents.

   (d) Flame photometry.

   (e) Sulphaemoglobin.

   (f) Reducing substances in urine.

   (g) Potassium dihydrogen phthalate.

   (h) Estimation of serum bilirubin.

   (i) Estimation of C.S.F. protein.

Wednesday, 7th March, 1962.

2.30 p.m.-5.30 p.m.

WRITTEN PAPER III—BACTERIOLOGY

Time allowed, three hours.

Answer ALL Questions.

1. (a) A nurse delivers to your laboratory a blood bank bottle containing about 200 mls of blood. It was stated that sometime previously this particular blood transfusion was discontinued, after 300 mls had been given, because the patient had a severe rigor followed by collapse. You are asked to examine this blood for possible bacterial contamination.

   Write a detailed account of:—

   (1) The information you should obtain concerning the history of this blood donation from the time it was collected until the time it was received by you. (10 marks.)

   (2) The bacteriological procedures you would carry out. (10 marks.)

1. (b) Comment briefly on the importance, if any, of Treponema pallidum, malarial parasites and the virus of infectious hepatitis in blood or plasma transfusion. (5 marks.)

2. Discuss the principles of steam sterilization. (10 marks.)

   If the theatre sister at your hospital thought that the theatre autoclave was inefficient how would you proceed to check its sterilizing efficiency? (5 marks.)

   If the autoclave was found to be inefficient you would need to check all aspects of autoclaving and the structure and installation of the autoclave. Outline how you would proceed to do this and indicate what parts of the autoclave are most likely to be faulty. (10 marks.)

3. (a) What is meant by the terms obligate anaerobe and facultative anaerobe? (5 marks.)

   (b) Describe in detail the structure and method of operating the anaerobic jar.

   (c) Describe one other method of anaerobic culture and state the principle on which it works. (15 marks for (b) and (c).)

   (d) What pathogenic anaerobic organisms might you expect to find in material from:—

   (1) A brain abscess.

   (2) A septic abortion.

   (3) A case of food poisoning. (5 marks.)

4. Write brief notes on:—

   (a) Transport medium.

   (c) Fimbrial antigens.

(c) Dye test for toxoplasma antibody.

(d) Haemolysin as used in the Wassermann Test.

(e) Penicillinase.

(Total 25 marks, 5 marks for each part.)

PRACTICAL PAPER I—BACTERIOLOGY.

Wednesday, 21st March.

9.0 a.m.-12 noon.

INSTRUCTIONS:

(1) State details of all methods and techniques used, including the temperature at which cultures are incubated.

(2) Retain all preparations which you make.

(3) Media and equipment will be provided on request if possible. If your requirements are not available the method you wished to use should be stated but wherever possible your method should be adapted to the material available.

(4) You may consult your text books.

1. Slide "A" is a fixed sputum smear prepared from the sputum of a patient with acute pneumonia.
Stain and examine this smear and give a full report on your findings. Write a brief account on how you would proceed to isolate and identify the organism from the sputum. (10 marks.)
(Pneumococci.)

2. Specimen "B" is a faecal concentrate. Examine this for the presence of ova and report what you find. How would you prepare a faecal concentrate suitable for the examination of ova. (10 marks.)
(Hookworm ova.)

3. Specimen "C" is a fluid obtained from a cystic structure. Examine the fluid microscopically, record what you find and state what the specimen is. (10 marks.)
(Hydatid cyst containing scolices.)

4. Culture "D" is a mixed culture obtained from a skin ulcer. Identify the organisms as far as you can by staining methods and make subcultures on appropriate media. State any additional tests which should be made. (30 marks.)
(S. aureus and C. diphtheriae.)

5. Cultures "E" and "F" are both staphylococcal cultures. Determine by both the slide and the tube tests if either culture is coagulase positive.
What substances are detected by the "slide" and the "tube" tests?
(Staphylococci—coagulase positive and negative.) (15 marks.)

6. Prepare a slide culture from the fungus culture "G" provided. (25 marks.)

DEPARTMENT OF HEALTH

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

PRACTICAL PAPER II—HAEMATOLOGY AND BLOOD BANK TECHNIQUE

Wednesday, 21st March.

2 p.m.-5 p.m.

1. Determine the group and Rhesus type of red cells supplied (labelled 1, 2 and 3).

   Test the compatability of each of these cell suspensions with the two sera X and Y, as for emergency cross-matching. (30 marks.)

2. Using the known red cells supplied (4, 5, 6, 7 and 8) identify the antibody present in the serum Z.

   Please see supplementary instructions provided. (20 marks.)

3. Examine the five stained blood films (A, B, C, D, E).

   Describe **any abnormal features** which you can see, make a differential leucocyte count only if you think it is necessary. Comments as to the probable diagnosis may be added if desired, but are not essential.
   (10 marks for each slide.)

   A. Nucleated red cells (Erythroblastosis).

   B. Normal blood.

   C. Untreated macrocytic ("pernicious") anaemia.

   D. Granulocytic leukaemia in a child 8 years, clinically a chronic leukaemia with gross splenomegaly, but there are some blast cells present.

   E. Excess of platelets.

PRACTICAL PAPER III—BIOCHEMISTRY.

Thursday, 22nd March.

9 a.m.-12 noon.

1. (a) Estimate the blood urea content of the specimen provided.
   (15 marks.)

   (b) Examine the urine provided for reducing substances and ketenes.
   (10 marks.)

2. (a) Estimate the calcium content of the serum provided using half an hour for precipitation. (15 marks.)

   (b) Write notes on each of the five "spots" provided. (25 marks.)

   (Palladium catalyst, Thermostat capsule, "A" grade 20 ml. pipette, Calomel electrode, Ammonium molybdate.)

3. (a) Estimate the diastase content of the urine provided. (10 marks.)

   (b) Write notes on each of the five "spots" provided. (25 marks.)

   (Petroleum ether 60°-80°C., Silver nitrate crystals, Bromosulphalein ampoule, Colloidal Gold Solution, Phenol Red Powder.)

   Methods to be used are those in the Standard Methods as circulated to all laboratories by the Health Department.

The following candidates were successful:

Mr K. Beckett, Auckland.
Miss A. C. Ellett, Auckland.
Miss D. Hall, Auckland.
Miss J. McClure, Auckland.
Miss J. M. McKenzie, Auckland.
Miss P. C. A. Somers, Auckland.
Mr R. Sowden, Auckland.
Mr D. N. Thorburn, Auckland.
Mis C. S. Curtis, Auckland.
Miss T. G. Logan, Hamilton.
Miss J. M. Wittam, Hamilton.
Mr P. A. Jones, Tauranga.
Miss S. M. McMullien, Whangarei.
Miss M. J. Stewart, Gisborne.
Miss A. K. Barraclough, Wellington.
Miss M. H. Burnett, Wellington.
Miss P. M. Lawn, Wellington.
Mrs B. M. Treadwell, Wellington.
Miss L. B. Wills, Wellington.
Miss S. A. Nielsen, Wanganui.
Miss A. Pridham, Palmerston North.
Mrs J. M. Taylor, New Plymouth.
Mr K. Boddy, Dunedin.
Mr D. A. Cathcart, Invercargill.
Mr J. E. Davies, Invercargill.
Mr T. J. Lewis, Nelson.
Miss F. E. S. Wright, Timaru.
Mr T. J. Naughton, Hastings.
Miss R. E. Samuels, Napier.

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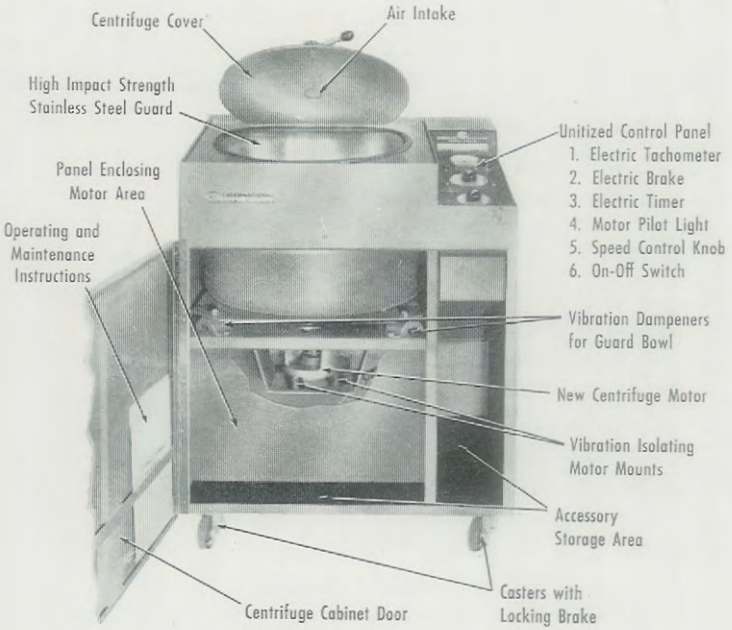

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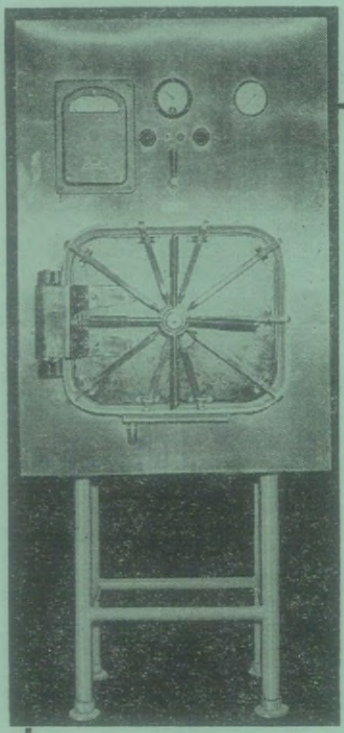

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