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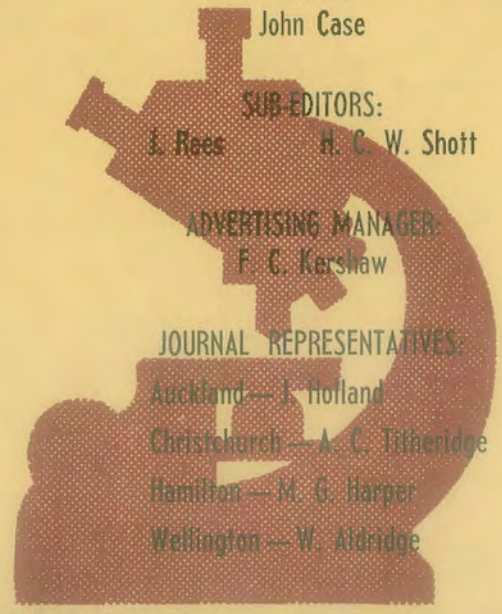

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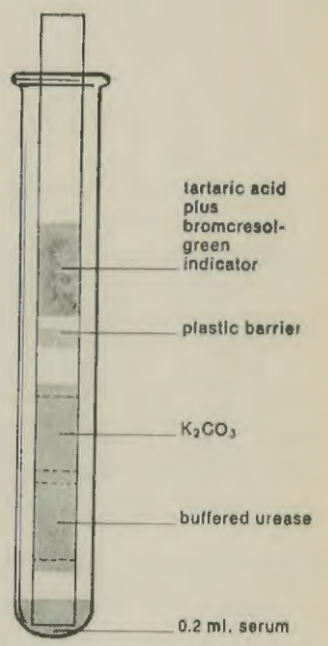

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Serum Urea Micro-Analysis: A Convenient Routine Method

Reagent Modifications in the Ammonia-Phenate-Hypochlorite Colour Reaction

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(Received for publication, July, 1964.)

In the field of urea analysis, attention has been drawn to the application of the colour reaction, first recorded by Berthelot (1859), involving a blue complex of ammonia, sodium phenate and hypochlorite [1 2 3 5 6]. The application of this colorimetric method has attributes and advantages as follows:

1. Sensitivity[7]. In the method outlined below, a urea concentration of 50mg./100ml. will give a corrected optical density greater than 0.6 at the wavelength of maximum extinction.
2. Stability. Developed colour may he safely left for 24 hours before determining optical density.
3. Interference. Interference by commonly encountered serum constituents is not clinically significant in the method as proposed [2 5].
4. Linear Urea Concentration/O.D. relationship at various wavelengths[2 5]. See study below.
5. Colour developed obeys Beer's Law.

In the proposed method, modifications in the distribution of reagent constituents have been aimed at eliminating entirely the difficulties of deterioration of urease and phenol-nitroprusside solutions of earlier methods[9]. The urease, sodium nitroprusside and di-sodium EDTA are retained in a stable dry mixture and dissolved immediately before use. This yields a urea method of application in the routine clinical laboratory.

In this respect, it is relevant to list points of significance:—

1. 20 μl. of specimen per test.
2. 10 ml. of final coloured solution is ample for all routine spectrophotometers.
3. Simplicity of technique.
4. Simple, inexpensive reagents.
5. Results compare closely with urea analyses performed by independent chemical analyses[5]. See comparison with *AutoAnalyzer* below.

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Principle

Urea in serum or plasma is hydrolysed with a buffered urease solution. The ammonium ions so produced form a blue complex compound with phenol and sodium hypochlorite in alkaline solution. Sodium nitroprusside is present as a catalyst[4]. Standard and blank determinations are included with each batch of serum analyses.

Reagents

1. *Buffered Urease-Nitroprusside.* (Renew every two months, and refrigerate when not in use.)

Di-sodium ethylene-diamine-tetra-acetate, 15
Sodium nitroprusside, 11 } parts by weight
Sigma type II Urease (from Sigma Chemical Company. 3500 DeKalb St., St. Louis 18, Missouri, U.S.A.). 2

The mixture is powdered and blended with a mortar and pestle and kept in a brown glass bottle. (Sodium nitroprusside is unstable in light.)

A 'spoon' (approx. 50 mg.) of this mixture is dissolved in 20 ml. distilled water immediately before use. (A suitable spoon may be made from glass or plastic.)

2. *1% Phenol*

5 g. phenol (analytical grade) dissolved in distilled water and made up to 500 ml.

3. *Alkaline Hypochlorite.* (Renew monthly.)

12.5 ml. of commercial sodium hypochlorite; 6.25 ml. of 20% NaOH; distilled water to 500 ml.

Sodium hypochlorite is available in solution from National Dairy Association of New Zealand. This solution contained 11% of available Cl^- (Iodometric Method as given by Vogel[5].)

4. *Primary Standard Solution* (Equivalent to urea concentration of 50mg./100ml.) 110 mg. of ammonium sulphate (AR) dissolved in distilled water and made up to 100 ml.

Secondary Standard (not essential).

Because of the lack of colour and the mobility of the aqueous standard in a Sahli pipette, it was found convenient in this laboratory, for ease of pipetting, to retain a bottle of quality control serum as routine standard, the urea concentration of this serum being checked weekly against the primary standard.

Procedure

1. 2 ml. aliquots of buffered urease-nitroprusside solution are pipetted into 6" x 1" test tubes of uniform wall thickness labelled for each serum, standard, blank.

2. Using a Sahli pipette, transfer 20 µl. of test sera or standard into the respective test tubes by rinsing the pipette *at least 6 times* in the urease solution. The blank does not require any addition.

3. Incubate the batch of tubes at 37°C. Time of incubation determined as in 1(a) below. (10 minutes for new urease.)

4. Add 4 ml. of 1% phenol solution followed by 4 ml. alkaline hypochlorite solution to each tube. Mix thoroughly. (These two solutions are conveniently dispensed from 3-way burettes with 2.5 litre reservoirs.)

5. Incubate the batch of tubes in boiling water bath for 5 minutes to develop colour.

6. Allow to cool to a satisfactory temperature for use in the spectrophotometer cuvettes. 1-2 ml. of solution poured to waste effectively removes water of condensation from wall of test tube before sample of solution is transferred to cuvette.

7. Read optical densities of tests, standard and blank at 670 mμ. If any tube of batch shows high optical density, test standard and blank may be read at 700 mμ for lower sensitivity.

Calculation

$$\frac{\text{O.D. Test} - \text{O.D. Blank}}{\text{O.D. Standard} - \text{O.D. Blank}} \times \text{Urea concentration of Standard} = \text{Serum Urea (mg./100ml.)}$$

Study of Factors Relevant to the Above Method.

1. *Optimum Concentrations of Reagents.*

(a) *Potency of Urease.*

An aqueous urea solution of concentration 300mg./100ml. was subjected to the proposed procedure in a series of determinations with varied time of incubation for hydrolysis. No further increase in optical density was obtained after 7½ minutes. To check that the optical density plateau so obtained was not due to the limiting factor of the quantity of reagent available for colour development, a double aliquot of urea solution (equivalent to 600mg./100ml.) was subjected to the proposed procedure. A further increase in optical density was obtained.

Conclusion: The method as proposed, with fresh reagents, will analyse sera of urea concentration up to 300mg./100ml. However, a limit of 250mg./100ml. should be stipulated to allow for the known slow decrease in urease potency over the 2 months specified as maximum age of the urease reagent at room temperature. (See 'Stability of Reagents' below.) For sera of urea concentration higher than 250mg./100ml. the determination should be carried out on a suitable dilution of the serum with water, and an appropriate calculation made.

Note: Newly purchased urease was used in this investigation. Since urease deteriorates slowly even at 4°C., a bi-monthly assessment of the hydrolysis time is essential. With decrease in urease potency, an increase in the time of hydrolysis is required. In the author's experience, after one year the required time of incubation has increased to 15 minutes.

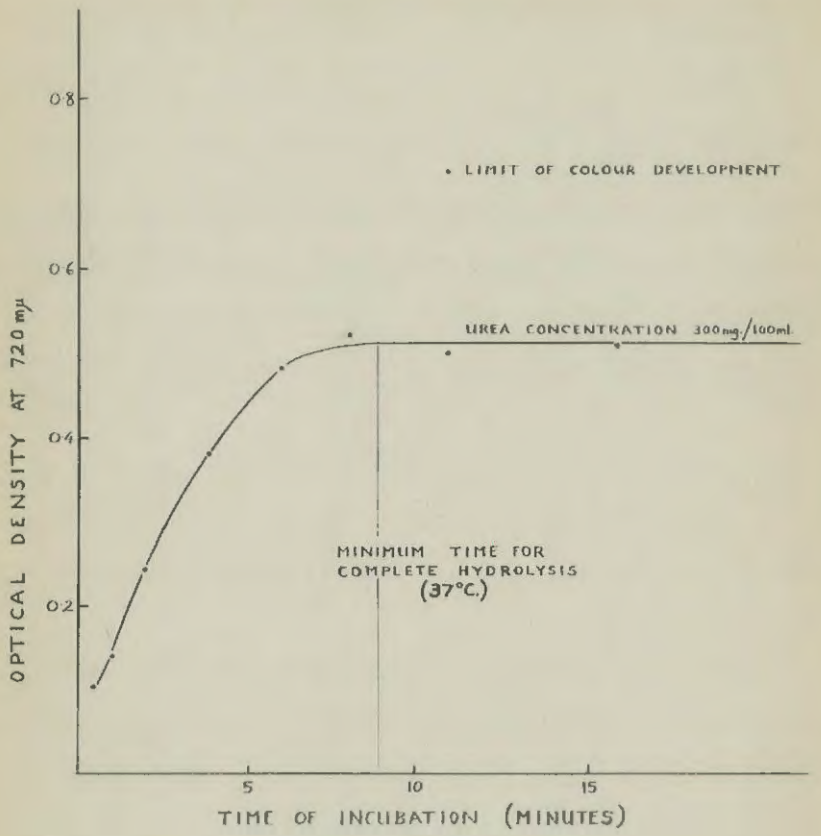

Fig. 1. Urea Hydrolysis.

(b) *di-sodium EDTA buffer.*

It was found that, during the hydrolysis stage, increase in alkalinity is detectable in unbuffered urease solutions. To counter any interference with the urease action by change in pH, a buffer is included. The di-sodium EDTA, in addition to serving as a buffer, is included in sufficient proportion as a 'bulking' agent for the urease and sodium nitroprusside.

(c) *Colour Development Reagents.*

It was noted that the blue complex is given only by certain relative concentrations of the reagents. An investigation was carried out to determine the optimum concentrations of reagents for maximum colour development because, given these optimum concentrations, the resulting optical density is less prone to

variability due to technical inaccuracy in volumes, or reagent deterioration. At the time of this study, a combined phenol-nitroprusside colour reagent as proposed by Searcy *et al.*[4] was being used. The scope of the investigation at this stage included variable concentrations of:

(1) Phenol Colour Reagent
(ii) Hypochlorite
(iii) Alkalinity

A series of experiments was undertaken in which the concentration of one reagent was varied while the concentrations of the remaining two reagents were constant. Initially, the concentrations of these latter two reagents were maintained as proposed, and then changed to the optimum concentrations as indicated by the immediately preceding experiments.

The final two experiments of this series are illustrated in Figs. 2 and 3. (Different ammonia concentrations used in each experiment).

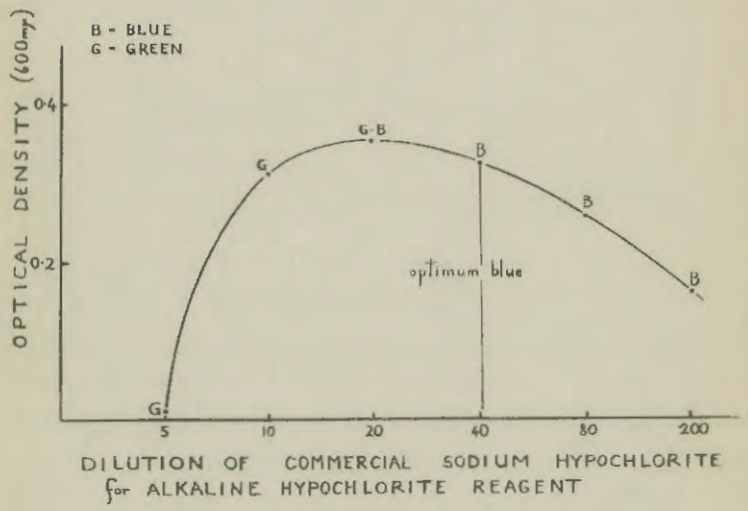

Fig. 2.

Later, with modification of the distribution of reagent components by transferring sodium nitroprusside from the phenol solution to the buffered urease, a similar investigation of this fourth variable, the nitroprusside, was carried out (See Fig. 4.)

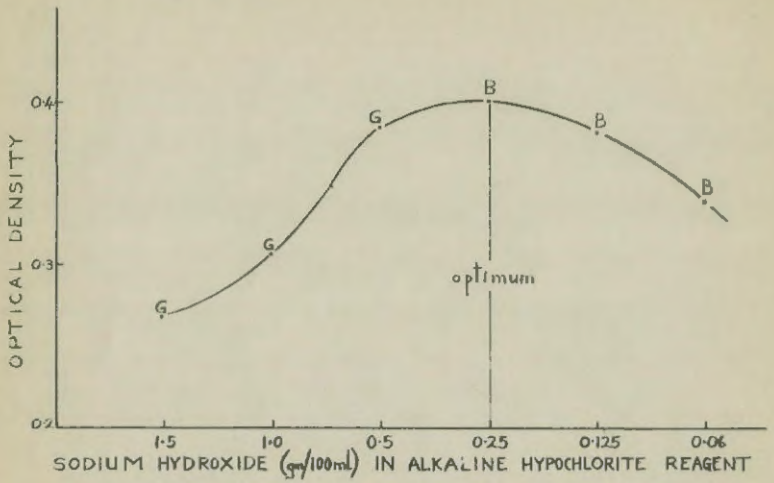

Fig. 3.

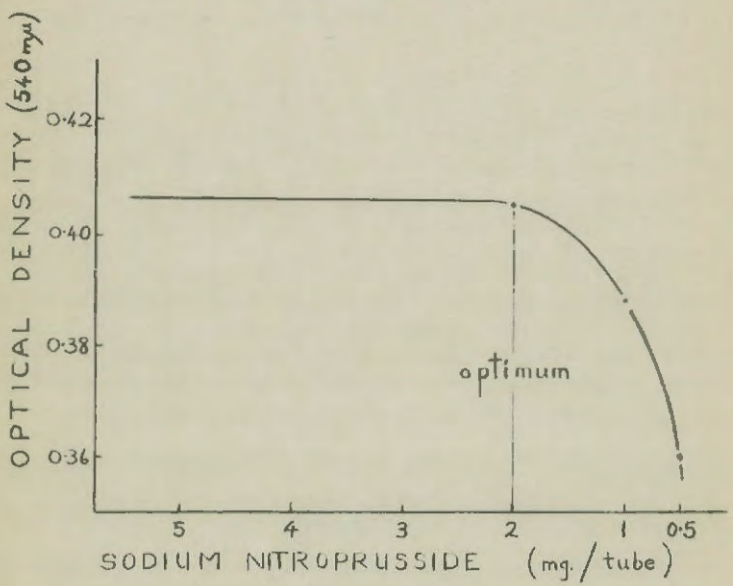

Fig. 4.

In addition, the optimum concentration of phenol alone was checked and found to be the same as originally indicated. See Fig. 5.)

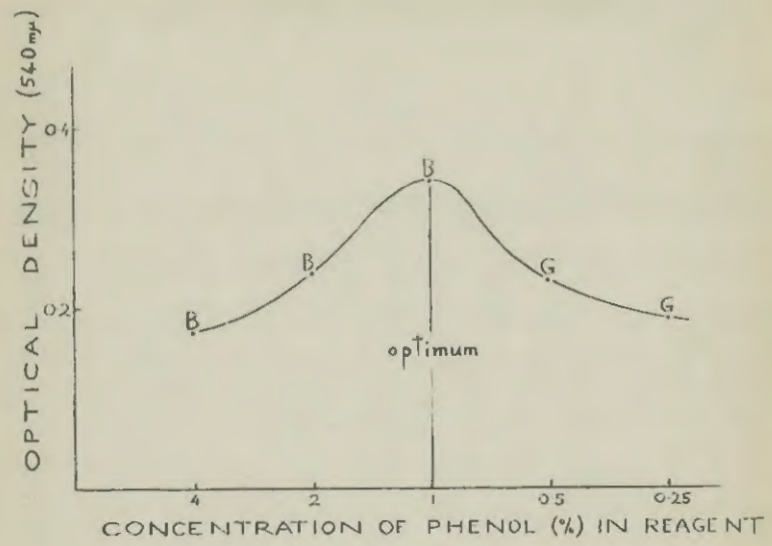

Fig. 5.

2. *Stability of Reagents.*

Urease solutions have only limited stability[2,5]. It was found that the use of a combined phenol-nitroprusside reagent involved increasing optical density of systematic reagent blanks with ageing of this reagent.

By retaining urease and sodium nitroprusside in the dry state, in a brown glass container, until dissolved for use, problems of stability of these components were markedly reduced. Urease potency deteriorates slowly at room temperature and it was found that after two months there was sufficient urease potency for the hydrolysis of sera of urea concentration up to approximately 250mg./100ml. It is advisable, therefore, to prepare only enough urease-nitroprusside-di-sodium EDTA mixture for 1 to 2 months usage.

The phenol reagent, therefore, consists of a simple phenol solution which has been found to be adequately stable in light at room temperature for at least two months.

The hypochlorite ion is reasonably stable in solution, and it has been the experience of this laboratory that the slow deterioration of the alkaline hypochlorite reagent at room temperature, resulting in slightly decreased colour development, is only of slight consequence in the proposed method, where test sera are related to a standard in each batch of analyses. (The actual decrease in optical density due to ageing of this reagent

for one month was in the region of 7% for the developed colour equivalent to a urea concentration of 50mg./100ml.). However, with ageing of the alkaline hypochlorite a small but detectable divergence from linearity in the colour response was noted, and for this reason a limit of one month on the age of this reagent should be adhered to.

In connection with the initial assessment of the concentration of commercial hypochlorite solution, it is noteworthy that J. K. Fawcett and J. E. Scott state that deterioration of hypochlorite solution, resulting in delayed colour development, is not accompanied by a parallel change in iodometric titre[2].

3. *Absorption Spectrum.*

Optical density/wavelength curves are illustrated in Fig. 6 for (a) blank, and (b) standard equivalent to urea concentration of 50mg./100ml.

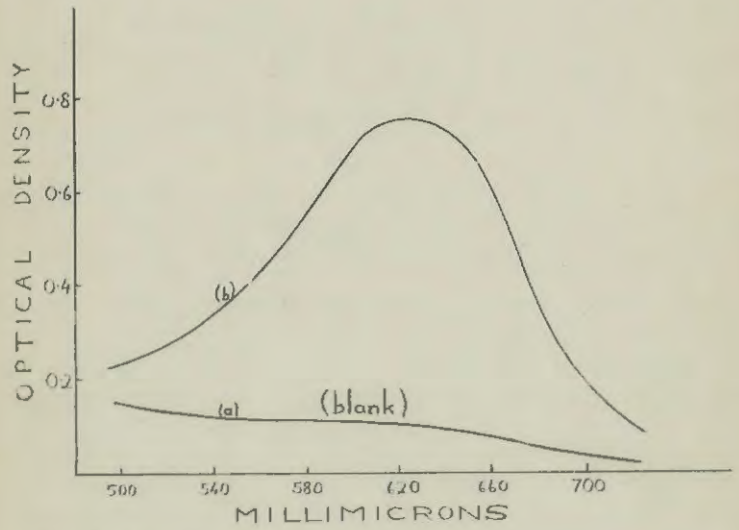

Fig. 6. Absorption Spectra.

The optical density maximum occurs at 630 mμ. Sensitivity of the method may be varied by selecting an appropriate wavelength for determining the optical density of the resulting solutions.

The optical density of the reagent blank decreases with increasing wavelength. It was proposed, therefore, to select wavelengths of suitable sensitivity which lie at greater wavelength than

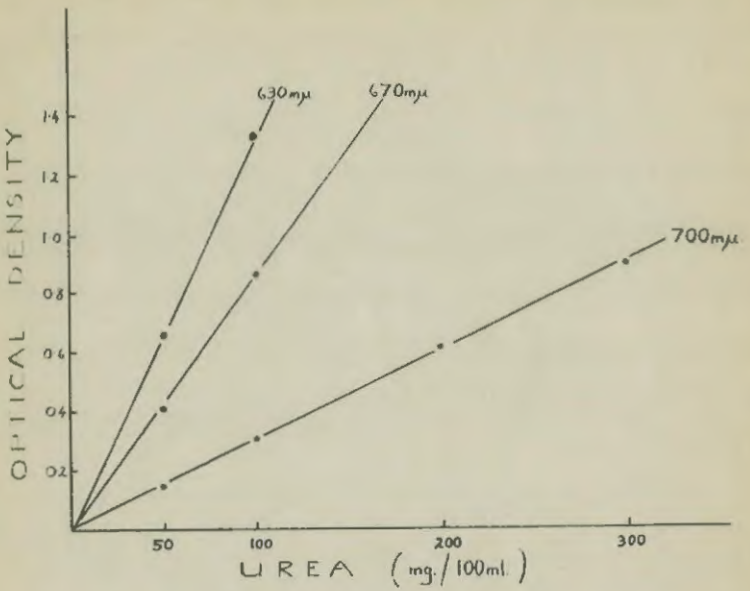

Fig. 7. Linear Optical Density/Urea Concentration Relationship.

that of the optical density maximum. The relationship between urea concentration and resulting optical density was shown to be linear at 630 mμ, 670 mμ and 700 mμ. (See Fig. 7)

4. *Stability of Colour Produced.*

Colour development is accelerated by increased temperature. With a view to speed, and the use of routinely available laboratory equipment, it was proposed to develop colour in the boiling water bath. With the 6″ x 1″ test tubes in use in this laboratory, solution temperatures reached 92°C. in 2½ minutes, and a total time of 5 minutes in the boiling water bath was found satisfactory for maximum colour development, without significant volume reduction by evaporation.

A survey of fading of the developed colour from a common dilution equivalent to 50 mg. urea/100ml. was conducted using reagents of differing age. The solutions were cooled for 5 minutes and then successive readings of optical density determined. (See Fig. 8.)

The following observations are made:

(a) Decrease in O.D. with ageing of the phenol solution is only about 1% in one month.

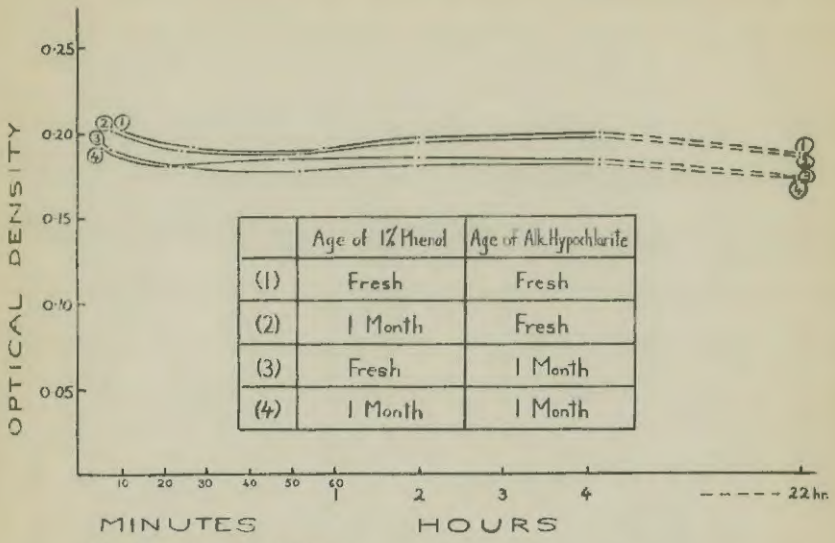

Fig. 8. Colour Stability.

(b) Decrease in O.D. with ageing of the alkaline hypochlorite reagent is about 7% in one month.

(c) rate of fading of colour developed is about 1% per minute 5 minutes after removal from boiling water bath, and negligible after 30 minutes.

(d) Change in O.D. from 30 minutes to 22 hours is less than 7%.

(e) The colour obtained varied slightly from bright blue to greenish blue, due to development of a background yellow colour with ageing of the alkaline hypochlorite reagent.

It is noted that with each batch of urea analyses, the inclusion of a standard which parallels the colour development and fading of the test solutions, adequately compensates for the small variations (a)-(e) above.

5. *Interference.*

In recent years, considerable investigation of possible sources of interference in methods similar in principle to that proposed has been carried out. It is useful to collate the information available:

(i) Glucosamine, glutamine, citrulline, which readily liberate ammonia, caused no interference[2].

(ii) Interference by haemoglobin and bilirubin is small, and the resulting decrease in accuracy is not clinically significant[2 5].

(iii) The anticoagulants sodium fluoride, potassium oxalate,

sodium citrate, heparin and liquoid, when used in concentrations four or five times those usually employed, did not cause any irregularity[3].

(iv) The common antibiotics penicillin, streptomycin, aureomycin, chloramphenicol and terramycin, when used in concentrations ten times greater than those in blood during the course of treatment, did not yield any observable inhibition[3].

Criticism of the sodium phenate method in relation to inhibition by streptomycin and chloramphenicol in a recent Australian urea analysis survey[5], in which reference is given to the work of P. Fleury and R. Eberhard[3], is worthy of elaboration. After stating that it was not possible to observe any inhibition with the antibiotics used in concentrations ten times greater than those in blood during treatment, Fleury and Eberhard observe that for concentrations a hundred times as great, an inhibition of 30-40% was noted for two of the antibiotics: streptomycin and chloramphenicol.

6. *Recovery.*

Results of an investigation of the recovery of added urea by the proposed method are given in Table I. The serum and plasma samples were from a variety of hospitalised patients.

Sample	Initial Urea Concentration Analysed mg./100ml.	Urea Added mg./100ml.	Final Urea Concentration Analysed mg./100ml.	Urea Recovered mg./100ml.	Recovery %
1.	28.2	65.2	91.4	63.2	97.0
2.	34.0	102.3	140.3	106.3	103.9
3.	34.6	102.2	136.6	102.0	99.8
4.	36.5	107.2	142.8	106.3	99.2
5.	140.8	55.3	194.4	53.6	97.0
6.	168.0	100.8	263.0	95.0	94.3
7.	188.0	99.9	275.9	87.9	88.0
				Average Recovery:	97.1%

Table 1

Although the recovery of case (7) is not as great as the preceding six, it is noteworthy that with urea concentrations of the high order measured in this instance (viz. 188, 276 mg./100ml.), experimental variation of only 2% in each of these values (184, 281mg./100ml.) could have yielded a 97% recovery.

Comparison with AutoAnalyzer Estimation

Serum and plasma urea values determined by the proposed procedure were compared with values determined by means of the *AutoAnalyzer* (diacetyl monoxime method.) The results are shown in Fig. 9.

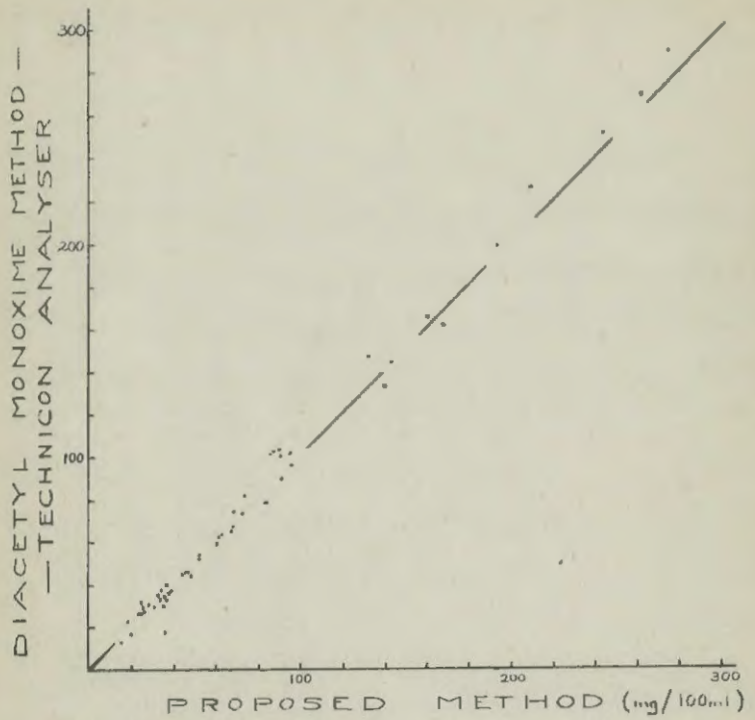

Fig. 9. Comparison of Methods.

Discussion.

Comparison was carried out on serum and plasma samples, including specimens of varying turbidity, age and concentration of bilirubin, haemoglobin and urea. The results from the *AutoAnalyzer* were chosen as suitable for comparison because of the established reproducibility of this unit. The diacetyl monoxime method measures molecular urea, whereas the proposed method effectively measures ammonia (resulting from hydrolysis of urea.) Age of specimens may be more significant, therefore, for results of diacetyl monoxime method than for the proposed method. 52 comparisons were carried out under routine conditions.

Reproducibility

Variability on this method may be related to
(i) Sampling (homogeneity of serum specimen)
(ii) Measurement of volume
(iii) Hydrolysis of urea
(iv) Colour Development

In connection with (ii) and (iv), systematic experiments gave relevant variation as follows:

(a) Technique related to reproducibility of pipetting (Sahli pipette), ± 2.4%.

(b) Measurement of volumes of colour development reagents and developing of colour in boiling water bath ± 1.25%.

In order to assess the overall reproducibility of the method, replicate analyses were performed on a sample of pooled sera having a urea concentration representative of a high normal urea level, where accuracy is of most clinical significance. Twenty-five analyses were performed under routine conditions. From an initial assessment of urease potency for a urea concentration of 200mg./100ml., the incubation time was set at 15 minutes at 37°C. Optical densities were read on a Bausch and Lomb "Spectronic 20" Spectrocolorimeter, the same cuvettes being retained for blank, standard and test respectively in each determination.

Results:

Mean Urea Concentration:		38.8	mg./100 ml.
Standard Deviation	: s =	1.07	mg./100 ml.
95% Confidence Limits	: ± 2s = ±	2.1	mg./100 ml.

Summary

A convenient routine micro-method for serum urea analysis, using the ammonia-phenate-hypochlorite colour reaction, is described. The proposed distribution of reagent components provides adequate reagent stability. A study of factors relevant to the method and reagents is outlined.

Acknowledgments

I wish to thank Dr F. H. Sims, Chemical Pathologist, for helpful advice. Thanks are also due to those of the staff at the Auckland Hospital Laboratories who have provided technical assistance.

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Uric Acid in Urine

A practical accurate method.

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The estimation of urinary urate excretion can be of considerable importance in many conditions and the relative disuse of this estimation has been partly due to the lack of a method which is accurate, yet simple enough for a general clinical laboratory.

Direct colorimetric methods (such as are used on serum) cannot be applied to urine due to the presence of varying and often large quantities of non-urate chromogens (N.U.C.). These N.U.C. levels have been observed here to vary anything between 10-120% of the urate concentration. The enzymatic, spectrophotometric method of Praetorius is specific, and the one of choice, but hardly suitable for routine use in a general laboratory, an ultra-violet spectrophotometer being essential and considerable experience necessary to achieve nice reproducibility and accuracy.

At this hospital, the daily urate excretion and the urate creatinine clearance ratio are important indices in the investigation and drug treatment of both metabolic and renal gout.

We have developed a simple and reliable method of determining the urine urate concentration, using the reagents already prepared for our existing serum method (Beale[1]). Purified Uricase* and a glycine buffer are the only added requirements.

The method is described and results compared with those obtained using the Praetorius method.

Principle of the Method

Urine is diluted in glycine buffer and the sum of urate and non-urate chromogens estimated by a direct colour development.

A portion of the diluted urine is incubated with the enzyme uricase at room temperature and, when the destruction of uric acid is complete, the remaining non-urate chromogens are estimated; this answer subtracted from the total represents the true urate content of the urine.

Other workers have used this principle: in serum (Block and Geib[2]); in serum and urine (Yu and Gutman[4]), (Buchanan *et al*[5]).

*Leo Uricase (75 units per ampoule).

Reagents

0.6 N NaOH

A convenient method of preparing this is to use a commercially preposed standard caustic solution. A litre of N NaOH can then be diluted to 1,666ml. with distilled water. Store in polyethylene or 'Pyrex' bottles.

Phosphotungstic Acid Reagent

Place 100 grams sodium tungstate (Merck G.R.) ($Na_2WO_4\ 2H_2O$) and 800ml. distilled water in a Florence flask, add 80ml. of Analar phosphoric acid (S.G. 1.75), boil gently under a reflux condenser for 2 hours, cool. Make up to 1 litre with distilled water and store in a brown bottle.

Glycine Buffer pH 9.4

Stock

Dissolve 12.5g. of glycine in 100ml. of distilled water. Add 55ml. of N. sodium hydroxide and make up to 250ml. Check pH and adjust to 9.4 if necessary. Add 1ml. of chloroform as a preservative and store the buffer in the refrigerator in a Pyrex bottle.

Working Solution

100ml. of stock is diluted to 1 litre with distilled water. This will keep a few days at room temperature but is best kept in the refrigerator.

Leo Uricase Solution

Measure out 2.5ml. of the working glycine buffer into a test tube; with a pasteur pipette transfer this into a 75 unit ampoule of Uricase and stand for 15 minutes. This can then be transferred back into the test tube for convenient handling. It is essential to be very careful with this solution; uricase is a potent enzyme, and contamination of fingers, or glassware, must be avoided.

Uric Acid Standard

Stock

Dissolve 2.3g. anhydrous sodium phosphate AnalaR (Na_2PO_4) in 300ml. warm distilled water. Transfer 100 mg. B.D.H. uric acid to a volumetric flask with the sodium phosphate solution. When the uric acid is dissolved, make up the volume to 500ml. with distilled water.

Working Standard

Make a 1 in 20 dilution of the stock standard in water, dispense immediately into C.O.C. plastic capped vials, keep deep frozen until immediately prior to use.

Take 3ml. of working standard, add 0.2ml. phosphotungstic acid reagent, mix, add 1.0ml. 0.6 N NaOH and read optical density at 15 minutes, as under procedure. This equals a 30mg./100ml. standard.

Collection of Urine

The patient is provided with a two-litre bottle containing

10ml. of toluene, and an instruction sheet. This tells him to empty the bladder, to discard this urine, and write down the time.

All the subsequent urines are voided directly into the bottle by means of a large funnel.

Approximately 24 hours after the time noted at the beginning of the collection, the patient again empties the bladder, this specimen goes into the bottle and the time is written down.

The interval, in minutes, between times noted is the exact collection time (T) to be used in subsequent calculations; (important in renal and metabolic studies).

Procedure

Bring the urine to pH 7.0 by the addition of caustic tablets, using Reidel de Haen special indicator paper (pH 5-9) and dilute 1 in 10 (1ml. urine in 9ml. glycine buffer working solution).

Into each of 2 tubes place 1ml. of the diluted urine, and mark one 'Total,' the other 'N.U.C.' To the 'Total' tube add 2ml. of glycine buffer; to the 'N.U.C.' tube add 1.4ml. of glycine buffer and 0.6ml. of the Uricase solution. Stand both tubes for 1 hour at room temperature.

At the end of this time add 0.2ml. of phosphotungstic acid reagent, mix by tapping, then add 1.0ml. of 0.6 N NaOH; mix by tapping. Exactly 15 minutes after the addition of the NaOH read optical density (O.D.) using a wavelength of 720 mμ on the Beckman Du spectrophotometer. 700 mμ is a satisfactory wavelength on less sensitive instruments, and the Hilger Biochem absorptiometer H810 (filter 70) has been found quite satisfactory.

When setting up the test prepare a total blank, an enzyme blank and a standard graph. The reagents, stored properly, are remarkably stable; and it is only necessary to check blanks and standard occasionally

Total Blank

3 ml. glycine buffer.
0.2 ml. phosphotungstic acid reagent.
Mix and add:
1.0 ml. 0.6 N NaOH.
Mix and read at 15 minutes.

Enzyme Blank

2.4 ml. glycine buffer.
0.6 ml. enzyme in buffer.
0.2 ml. phosphotungstic acid reagent.
Mix and add:
1.0 ml. 0.6 N NaOH.
Mix and read at 15 minutes.

The colour developed, read in the Beckman Du Spectrophotometer at 720 mμ, has a straight line standard graph.

It is our practice to do a quick preliminary total estimation omitting the hour standing, and if the optical density of the total chromogen tube is over 0.500 a greater dilution of the urine is made (e.g. 0.5 ml. urine in 9.5 ml. glycine buffer), adjusting the final result accordingly.

Calculation

1. O.D. 'total' minus O.D. 'total blank'.
2. O.D. 'N.U.C.' tube minus O.D. 'enzyme blank' (look up each on standard graph), 2 being the non urate chromogen content, the difference between 1 and 2 gives the true uric acid concentration per 100 ml. (U).

This figure (U) in conjunction with the urine volume (V) in ml., the period of collection in minutes (T), and serum uric acid level (P), is used to calculate urate clearance and urate excretion figures from the formulae:

$$\frac{UV}{PT} = \text{ml. per minute urate clearance.}$$

$$\frac{U \times V}{T} = \text{mg. excreted per 100 minutes.}$$

Accuracy

To ascertain the reproducibility and validity of the method, 21 urines were examined by this and the Praetorius method (Table I).

CASE	TOTAL URATE + N.U.C.	N.U.C.	COMBINED ENZYMATIC COLORIMETRIC METHOD mg./100ml.	PRAETORIUS METHOD mg./100ml.
A	42.6	12.6	30.0	29.7
B	52.8	12.6	40.2	36.2
C	44.1	5.4	38.7	37.5
D	46.2	4.8	41.4	41.8
E	50.5	10.2	40.3	40.4
F	44.4	12.0	32.4	31.0
G	54.0	10.8	43.2	43.5
H	60.0	13.2	46.8	46.8
I	30.1	5.5	24.6	25.2
J	48.0	11.0	37.0	36.0
K	22.5	8.4	14.1	14.4
L	48.0	22.2	25.8	28.7
M	57.9	12.0	45.9	45.6
N	20.2	3.7	16.5	17.0
O	28.2	4.8	23.4	23.4
P	18.0	4.2	13.8	13.2
Q	98.4	30.0	68.4	68.1
R	33.0	18.0	15.0	16.2
S	47.1	8.4	38.7	36.5
T	63.6	22.8	40.8	40.8
U	79.9	11.5	68.4	69.0

Table I. Uric acid concentrations on 21 urine speciments.

Several of these urine specimens contained large quantities of protein up to the level of 1 gram per cent. As protein does not affect the Praetorius method, these results show that there is no serious interference in the combined method, and protein removal is unnecessary, as well as undesirable.

Summary

A method for estimating urinary uric acid is described. This is simple, accurate, and within the scope of any clinical laboratory; the results correlate well with those obtained using the Praetorius enzymatic spectrophotometric method.

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The Hydatid Haemagglutination Test and Allied Techniques

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A variety of tests have been devised to detect antibody response to hydatid disease, notably complement fixation, haemagglutination, bentonite flocculation and latex agglutination. Of these the haemagglutination test has received particular attention on the basis of its supposed specificity, sensitivity and ease of technical execution. The purpose of the investigations undertaken in this work has been to establish the haemagglutination test as the primary routine method for the clinical laboratory. A brief appraisal of other methods in current use may also give support to the contention that they are best suited to play a complementary role in merely detecting the presence of antibody, rather than providing a yardstick of antibody response.

The haemagglutination method described is essentially that of Boyden (1951), certain modifications having been introduced to enhance the reliability and reproducibility of results. The test depends on the adsorption of antigen onto the surface of sheep erythrocytes which have been previously modified by tannic acid. When the antigen adsorbed red cells are exposed to a related antibody they clump together to form a pattern of agglutinated cells. In the absence of agglutinins the cells form a compact button as the result of normal sedimentation. Being a quantitative serological test, not only is the detection of hydatid antibody possible, but the titration of such reaction may demonstrate a clinically significant titre associated with a recent antibody stimulus.

The Indirect Haemagglutination Test

Preparation of Reagents

(a) Tanning of Red Cells

To previously weighed tannic acid (0.01 gm. of powder in a Universal container) add 10 ml. of normal saline, mix; when dissolved a 1/1000 dilution of tannic acid is obtained. Transfer 0.5 ml. of this concentration to 19.5 ml. of normal saline, already delivered into a further Universal, thus on thorough mixing a 1/40,000 tannic acid solution is prepared. The actual tanning of the red cells is carried out in duplicate, one suspension for control purposes, the other for the test.

The tanning process is as follows:—

Remove 1 ml. of the 20 ml. of (1/40,000) tannic acid from each of the two Universal containers and replace with 1 ml. of thrice-washed, packed sheep cells. It is important that the cells

are 24 hours old before use, also that all cells are evenly suspended in the tannic acid solution. Placed in the 37°C. water bath the cells are exposed to the action of tannic acid for precisely 10 minutes, during which time they are gently agitated at 2½-minute intervals. Once treated with the tannic acid the cells are centrifuged at 1,500 r.p.m. for 3 minutes. The supernatant is then carefully removed and the deposited cells washed once in 20 ml. of normal saline. Finally one tanned cell deposit is re-suspended in 0.2% gelatine in normal saline, to be retained to provide the *tanned cell control.*

(b) The Sensitisation of the Tanned Cells.

The remaining deposit of tanned cells is resuspended in 4.5 ml. of saline. When all the cells are evenly distributed, 5 ml. of undiluted, pooled hydatid cyst fluid is added. To allow the cells to become sensitised they are left at room temperature for 25 minutes, with occasional shaking. The sensitised cells are centrifuged at 1,500 r.p.m. for 3 minutes, washed once in normal saline and then carefully resuspended in 0.2% gelatine in normal saline to the original volume of 20 ml.

Performance of the Test

(a) **Dilution of the Test and Control Sera.** To obtain an initial 1/20 dilution, take 0.5 ml. of previously inactivated serum and mix with 9.5 ml. of 0.2% solution of gelatine-saline. By means of the conventional doubling dilution technique (carried out in a W.H.O. perspex influenza tray), prepare a range of the serum dilutions, using a 0.5 ml. volume, from 1/20-1/10,240. Thus the ten wells per row will be occupied with serum dilutions from each respective test and control serum.

Having prepared all the intended test and control dilutions of sera, add 0.1 ml. of the sensitised cells to every well. Mix by careful rotation of the tray.

(b) **Control of Tanned and Sensitised Cell Suspensions.** Include the following:

1. First serum dilution of test and positive control sera, plus 0.1 ml. volume of normal, untreated cells in 0.2% gelatine.
2. First serum dilution of test and positive control sera, plus 0.1 ml. of suspension of tanned cells in gelatine.
3. Diluent (0.2% gelatine in saline), plus suspension of sensitised cells.
4. A negative serum titrated in full, positive likewise.

After leaving at room temperature for 3 hours the results are read, beginning with the set of controls, including the known positive serum. Results are valid if there is a complete absence of agglutination in all types of negative cell control, *i.e.* a compact button of cells confined to the bottom centre of the well. True agglutination at any particular dilution of serum may vary from coarse agglutination to a fine distribution of clumped cells covering the whole of the well floor.

The preference given to the latex rather than the H.C.F. is on the grounds of reproducibility and ease of technical manipulation. It is interesting to note that on no less than two occasions old calcified cysts have been associated with marginal titre results using both red cells and latex particles.

Experimentally, without any real success, an attempt was made to use a direct haemagglutination method, using pre-heated cyst fluid supernatant as the antigen. Absorption of sera (giving low haemagglutination titres) with unsensitised sheep cells, human liver and kidney tissue, gave rise to speculation that Forsmann antibodies, or possible manifestations of auto-immune response, may give rise to low or marginal haemagglutination and latex titres. Much has still to be learnt regarding the optimum concentration of antigen used to sensitise both cells and particles alike.

In the early phase of antibody response, it may be difficult to obtain a reaction. Marginal results and false positive reactions are caused by the non-specificity of antigens. The association between blood group P substance in hydatid cyst fluid (Cameron and Staveley, 1957)[2] probably has little bearing on the overall problems.

Interpretation Of Results

The demonstration of a haemagglutination titre of 1 in 320 or greater, supported by a 1 in 16 latex agglutination, may be considered diagnostic. Anything less than these findings would call for careful assessment on a clinical basis.

Summary

Technical details of the hydatid haemagglutination test have been described in detail. An attempt has been made to present the method, together with its advantages and disadvantages. There seems little doubt that no single test is consistently diagnostic, some evidence has been given to co-ordinate the haemagglutination and latex results as a rational approach to the problem of laboratory diagnosis of hydatid disease. Meanwhile the standardisation of a control serum and antigen on a national basis might well remove some of the existing problems.

Acknowledgments

I wish to thank Professor J. A. R. Miles of the Microbiology Department, University of Otago, for permission and facilities to undertake this work.

To Associate Professor N. P. Markham I am indebted for his advice and guidance; also to Dr K. Moriarty who gave me initial help.

I further acknowledge the receipt of sera from the various diagnostic laboratories throughout New Zealand. I would also like to thank Miss M. Veitch for her willing technical assistance.

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An Indirect Micro Test for L.E. Cells

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Since the description of the lupus erythematosus cell (L.E. cell) by Hargraves, Richmond and Morton in 1948[2], testing for L.E. cells has become a routine procedure in most clinical laboratories. The L.E. cell factor acts on nucleoprotein (derived from leucocyte nuclei) which is subsequently ingested, probably with the aid of complement [1 5], by phagocytes — commonly neutrophil polymorphonuclear leucocytes[2]. Techniques for the demonstration of these cells fall in general into direct or indirect methods. In direct methods the test blood provides, in addition to the L.E. cell factor itself, the other components involved. An indirect test, on the other hand, merely requires the test serum or plasma to provide the L.E. cell factor, with the other components being supplied from elsewhere. A technique using buffy layers obtained from blood in heparinized capillary tubes for both direct and indirect testing has been described[6]. For indirect testing it is indicated that two or three buffy layers should be mixed with a drop of test serum or plasma, followed by incubation and smearing. As it is apparent that it is precisely at the point of mixing of test serum or plasma with 'substrate' that small differences in technique can profoundly influence the yield of L.E. cells, various methods of mixing have been examined which might provide optimum conditions for the formation of these cells. The following method utilizes freshly drawn blood in plain capillary tubes as a source of the accessory factors.

Technique

Materials

In addition to routine laboratory equipment, a glazed or siliconized agglutination tile, wooden applicator sticks and some means for drawing up and expelling the contents of varying lengths of capillary tubing is required. (See Figure).

Method

1. Normal capillary blood from a freely flowing skin puncture is drawn up into unheparinized capillary tubes (75mm. x 1.3-1.5mm.) which, within a few minutes of withdrawal, are sealed and centrifuged as for haematocrit. This will form the 'substrate.' Freshly drawn venous blood prior to coagulation can

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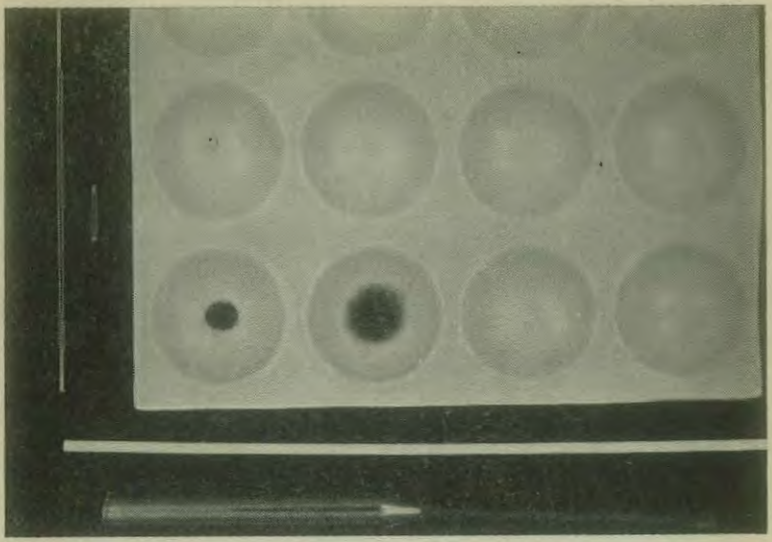

The capillary tube (extreme left) contains the test serum. Excess serum and red cells have been cut away from the leucocyte layer in the adjacent centrifuged tube of freshly drawn blood to form the 'substrate.' A short length of commercial bicycle valve rubber attached to a suitable mouth piece (below) and evaginated at the tip is suitable for drawing up or expelling the contents of fine capillary tubing. Also shown is a wooden applicator stick and the agglutination tile. A cup in the tile (extreme lower left) contains the 'substrate' and the adjacent cup has had the test serum added followed by crushing of the leucocyte button prior to incubation of the preparation (see text).

also be used if a large number of tests are being carried out. Some form of plastic sealing is preferable to flame sealing, so that no burnt material can form to interfere with the test.

2. Up to about 15mm. of the red cell column and up to about 10mm. of the serum column is cut away from the leucocyte layer and blown out (serum first) into a cup in the agglutination tile. A fibrin strand which may be formed in the serum column can be severed with the cut edges of the tube. Up to two-thirds of a capillary tube (50mm.) of test serum is now added to the cup. If more than about four tests are being performed, the remainder are covered to prevent evaporation while the next steps are in progress.

3. The button of leucocytes is carefully crushed with the end of the applicator stick about thirty times (or until numerous small fragments can be seen) with short rapid vertical movements — the button generally adheres to the tip of the stick and is actually withdrawn from the fluid with each stroke. The preparation is effectively mixed during this process.

4. The mixture is now drawn up into a plain capillary tube picking up as many as possible of the leucocyte clumps (especially from the periphery of the preparation) which might otherwise be left adhering to the cup.

5. After sealing, the tube is incubated at 37°C. for 45 minutes and then centrifuged as for haematocrit. All but a few mm. of the red cell column and serum column is cut away from the leucocyte layer which is then expelled and mixed on a slide followed by smearing and staining as for blood films.

6. The whole of the preparation is scrutinized for L.E. cells, especially at the edges and tail of the film.

Discussion

Some distinct advantages can be gained by indirect testing. Fresh test blood is not required and, as the L.E. cell factor readily stores frozen for indefinite periods, retrospective studies can be undertaken. In addition, as all the accessory factors are supplied at the time of testing, materials other than whole blood, serum or plasma can be tested for L.E. cell factor activity. The inhibitory factors to L.E. cell formation[1,5], occasionally found on direct testing, may be overcome by the addition of fresh normal 'substrate' to the system.

In the technique described here, the results generally appear at least as 'positive' as the routine direct tests used, and often a much higher proportion of L.E. cells can be found. One slide per test is normally prepared and examined. Only cells containing the characteristic inclusions, as originally described, are considered diagnostic and these should be differentiated from tart cells[2] which, unless recognised, can lead to error.

Within limits the proportions of the reagents employed can be varied. The more test serum used the greater the sensitivity. If the proportions are kept constant the test can be made approximately quantitative with respect to the L.E. cell factor activity of the test material. Finer scoring than numerous L.E. cells '3+', moderate numbers L.E. cells '2+', and few L.E. cells '1+' is not attempted. In a '3+' rating numerous L.E. cells are easily found often forming numbers of varying sized groups; in a '1+' rating only an occasional L.E. cell is found and with some difficulty; while a '2+' rating is intermediate. Rating '±' is indeterminate and is used when isolated non-phagocytosed altered nucleoprotein is found, or when 'rosettes' comprising numbers of leucocytes surrounding similar masses are seen. In these circumstances repeated testing, with variation in the proportion of test serum to normal 'substrate' serum is indicated.

Heparinized capillary tubes can be used, although the yield of L.E. cells appears somewhat less. The dispersion of leucocytes,

the so-called anticomplementary nature of heparin and the inhibition of coagulation, may be factors of importance.

The effect of crushing the leucocyte button with the wooden applicator stick is probably partly due, at least, to the traumatization of the leucocytes. This could result in an increase in dead leucocytes providing further 'substrate' nucleoprotein. The use of freshly drawn blood without anticoagulant ensures maximal activity of the surviving leucocytes and complement.

The small quantities of test serum used and the apparent sensitivity of the method are valuable assets in the study of the mouse L.E. cell factor[3 1], where supplies of serum are limited and for which the test was originally developed. These same factors may render the test of practical value in the clinical laboratory.

Acknowledgments

I am indebted to Associate Professor J. B. Howie and Mr J. Rees for technical assistance and helpful criticism. Thanks are due to various individuals who have sent me specimens of serum from patients with S.L.E.

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Selected Abstracts

(Contribution to this issue: R. D. Allan, J. Case, H. C. W. Shott and D. Tingle).

BLOOD BANKING

The Influence of Albumin in the Antiglobulin Crossmatch. Griffiths, J. J.; Frank, Sally and Schmidt, R. Pauline. (1964), *Transfusion (Philad.)*, **4**, 461.

From a study of 453 antibodies, it is concluded that bovine albumin, in a final concentration of 17% in the incubating mixture in the indirect antiglobulin test, enhances the sensitivity of the technique. Not only does the presence of albumin strengthen or enhance reactions, but some antibodies are revealed which are not detectable by the conventional saline antiglobulin test.

Lutheran^b Isoimmunization in Pregnancy. Herrick, C. N. Ladner, C. N., Pearson, J. W. and Harrison, H.E. (1964), *Obstet. Gynec.*, **24**, 855.

This is a report of a case in which the uncommon antibody anti-Lu^b was discovered in the serum of a pregnant woman. The pregnancy was allowed to proceed to term on the grounds that no case of haemolytic disease of the newborn due to this antibody had been reported, and when eventually delivered, the infant was found to have a negative direct antiglobulin test, in spite of being Lu^b positive. There was no free anti-Lu^b in the cord serum.

The D^u Crossmatch. DeWitt, T. H. Jnr. and Holland, Kathryn F. A. (1965), *Amer. J. clin. Path.*, **43**, 142.

As a safeguard against the inadvertent administration of D^u blood to rhesus negative recipients, these authors are suggesting a modification of the crossmatch in rhesus negative cases, in which a potent anti-D serum is added to the recipient's serum for the performance of the indirect antiglobulin crossmatch.

A Simple Serological Test for Antibodies Causing ABO Haemolytic Disease of the Newborn. Polley, Margaret; Mollison, P.L.; Rose, Jane and Walker, W. (1965), *Lancet*, **1**, 291.

A serological method, based on Witebsky's partial neutralisation test, was used to estimate the amount of gamma-globulin anti-A (or anti-B) in thirty-three cases of suspected ABO haemolytic disease. In all but one case, gamma-globulin antibody could be estimated with a titre in the range 64-16,000.

The titre was 1,000 or more in thirteen out of eighteen cases needing exchange transfusion, and in four out of fifteen that did not.

In a control series of sixteen apparently healthy group A infants born to group O mothers, the maternal gamma-globulin anti-A was 1,000 in 1 case and 128 in another, but in the remaining fourteen it was in the range 0-64.

Short Term Storage of Enzyme-Treated Cells. Webb, D. H. (1964), *Vox Sang. (Basel)*, **9**, 510.

Red cells, collected in ACD anticoagulant, ficinised within forty-eight hours of collection, then well washed and stored as a 10-20% suspension in 2.5% sodium benzoate at 4-6°C. will keep for up to 14 days. To prepare for use, the cells require washing at least once in normal saline.

A Scheme for the Ante-Natal Prediction of ABO Haemolytic Disease of the Newborn. Ames, A. C. and Lloyd, R. S. (1964), *Vox Sang (Basel)*, **9**, 712.

The suggested scheme is to test any serum showing lysis of group A or group B cells at room temperature by a modified antiglobulin test against group A or group B cells. (The serum is partially neutralised by the addition of a tenth volume of A & B substance before incubating with A

or B cells). If positive, the husband's group is determined and if there is an ABO incompatibility between the husband and wife, arrangements are made in preparation for a possible affected baby.

In the series studied, 260 out of 8,000 women had positive modified antiglobulin tests. In 116 the patient was ABO incompatible with the husband and a clinical diagnosis of haemolytic disease was made on 24 infants born to women in this group. Three were exchange transfused.

CHEMICAL PATHOLOGY

Rapid Stick Method for Determining Blood Glucose Concentration. Marks, V. and Dawson, A. (1965), *Brit. med. J.* i, 293.

Experience with the Dextrostix method of determining blood glucose is described. There is good agreement with conventional methods of blood glucose estimation in the normal and hypoglycaemic range, and the technique is useful for recognising but not quantitating blood glucose concentration in the hyperglycaemic range.

Rapid Estimation of Urea in Whole Blood with Urastrat. Baron, D. W. and Hughes, G. (1965), *Brit. med. J.*, i, 233.

By adding a mixed solution of dextran, phytohaemagglutinin and heparin to whole blood in equal proportions, rapid firm agglutination is achieved and after ten minutes separation, Urastrat urea estimation is possible at the bedside, with an accuracy of $\pm$ 20% in the 30-500 mg./100 ml. range.

Influence of Iron Preparations on Occult Blood Tests. Illingworth, D. G., (1965), *J. clin. Path.*, **18**, 103.

A variety of results are obtainable depending on the nature of the preparation and the test employed. Traditional iron remedies such as ferrous sulphate and citrate do not interfere, but newer preparations such as ferrous fumarate (Fermasal) and ferrous carbonate (Ferrodic) give positive results with haematest, occultest and benzidine. Ferrous amino-aceto sulphate (Plesmat) gives a positive benzidine but a negative orthotolidine. The results refer to filter paper techniques, benzidine tube tests do not give false positives. R.D.A.

Spectrometric Determination of Abnormal Haemoglobin Pigments in Blood. Martinek, R. G., (1965), *Clin. chim. Acta*, **11**, 146.

The author remarks on the scarcity of articles bearing on direct methods for estimating abnormal haemoglobins.

The methods described are for two component systems, *i.e.* Hb 0 with Hb unit Hb S or Hb CO. The presence of only one abnormal pigment must be determined with the Hartridge Reversion Spectroscope. Except for the use of a buffer for Hb CO, analysis is accomplished by measuring absorbancy of diluted blood at the isobestic point and at a wavelength where the densities are widely separated.

Details of constants determination are given. R.D.A.

A Simple Method of Serum Protein Fractionation of Cellulose Acetate and a Comparison of the Albumin Levels with a Method of Sodium Sulphite Fractionation. Webster, D. (1965), *Clin. chim. Acta*, **11**, 101.

The general technique employed was that described in the Oxoid notes for using cellulose acetate strips. The author found good agreement between C.A. electrophoresis and fractionation with 28% sodium sulphite in normal sera but not when albumin is low and globulin high. In such cases electrophoresis is advocated.

Two interesting points are:

1. The use of 10% Teepol for eluting the fractions. Optical densities are the same as using sodium hydroxide and acidifying with acetic.
2. A comparison of the dye uptake of albumin and gamma globulin using Ponceau S shows that the globulin dye uptake is about two-thirds of the albumin. The actual ratio of optical densities is 1.57.

R.D.A.

The Kinetic Spectrophotometric Assay for Serum Alkaline Phosphatase. Frajola, W. J., Williams, R. D. and Austrad, Ruth S. (1965), *Amer. J. clin. Path.*, **43**, 261.

A comparison is made between results using a conventional Bessey Lowry method and the change in optical density obtained by a recording spectrophotometer. The unit is based on the linear change in density. In practice, this calls for very few manipulations and is simpler than the conventional technique. No blank reading or subtraction is required. There was good comparison. R.D.A.

HAEMATOLOGY

Correction for Trapped Plasma in Microhematocrit Determinations. Rustad, H. (1964), *Scand. J. clin. Lab. Invest.*, **16**, 677.

The volume of plasma trapped in the packed red cell column in capillary tube haematocrit determinations has been measured by radioactive iodine-tagged serum albumin.

An average overestimate of $2.78 \pm 0.11\%$ of the read haematocrit value was found.

The Westergren Sedimentation Rate Using K_3EDTA. Gambino, S. R., DiRe, J. J., Monteleone, Marianne and Budd, D. C. (1965), *Amer. J. clin. Path.*, **43**, 173.

From the results of this study, it is concluded that tri-potassium EDTA is a suitable anticoagulant for the E.S.R. provided the anticoagulated blood is diluted four parts with one part of saline for the test. In addition, it was shown that K_3EDTA blood can be stored for twelve hours at 4°C. without a significant change in the E.S.R., and that the Westergren test is technically and clinically superior to the Wintrobe test.

A Source of Error in the Cyanmethheoglobin Method of Determination of Hemoglobin Concentration in Blood Containing Carbon Monoxide. Taylor, J. D. and Miller, J. D. M. (1965), *Amer. J. clin Path.*, **43**, 265.

The reaction of carboxyhaemoglobin with conventional Drabkin's reagent is much slower than the reaction with oxyhaemoglobin. When measuring total haemoglobin concentration in bloods containing carboxyhaemoglobin, the time of reaction before measuring optical density must be prolonged or, alternatively, a special reagent containing five times the concentration of ferricyanide must be used.

Diagnostic Value of Serum Haptoglobin. Shinton, N. K., Richardson, R. W. and Williams, J. D. F. (1965), *J. clin. Path.*, **18**, 114.

Serum haptoglobin was estimated quantitatively in 25 patients with haemolytic anaemia, 110 normals, 149 patients with other forms of anaemia and 37 patients with non-haematological disorders.

The normal range was found to be 33-213 mg./100 ml. Subnormal levels were found in 80% of patients with haemolytic disease or megaloblastic anaemia, patients with haemorrhage into tissues and occasionally in association with other diseases. The diagnostic value of the estimation of serum haptoglobin is discussed.

The Direct Antiglobulin (Coombs) Test in Megaloblastic Anaemia. Forshaw, J. and Harwood, Lilian. (1965). *J. clin. Path.*, **18**, 119.

Out of 32 patients with megaloblastic anaemia, ten gave a positive direct antiglobulin test. There was no colleration between the result of the test and the degree of anaemia, and no significant difference between the incidence of positive results associated with a deficiency of vitamin B_{12} or folic acid.

The Effect of the Use of Different Tissue Extracts on One Stage Prothrombin Times. Poller, L. (1964), *Acta Haemat*, **32**, 292.

The effect of the use of a variety of thromboplastin reagents on the results of one stage prothrombin time determinations was studied. These consisted of three human brain preparations, five animal preparations and

three reagents incorporating absorbed plasma (Withington, 2-7-10 and Thrombotest). Nine of the ten tissue extracts gave results of the same order. The three complex absorbed plasma reagents were alike in their results, but their therapeutic range appeared to be substantially lower in percentage activity than the simple tissue extracts.

The Assessment of Anticoagulant Therapy and Comparison of Quick Test, Thrombotest and 2.7.10 Reagents. Taylor, D. M., Stenbeck, C. L. and Erenstrom, A. J. (1965), *N.Z. med. J.*, **64**, 29.

This is a comparison of three methods of controlling anticoagulant therapy and a discussion of the implications of the findings.

The thrombotest and 2.7.10 methods, which are virtually identical, give much less inherent patient variability than the Quick test; but, in common with others, these workers have found that there may be a need to modify the therapeutic range to make thrombotest and 2.7.10 control acceptable to clinicians who have been accustomed to dosing their Quick-controlled patients more heavily without an undue incidence of haemorrhage.

Coagulation Tests in Anticoagulant Therapy. Davies, D. W. (1965), *Med. J. Aust.*, **i**, 150.

Three methods were used in parallel to evaluate the blood coagulation activities of a group of 189 patients receiving oral anticoagulant therapy. The methods were: thrombotest, the one stage prothrombin time and the kaolin partial thromboplastin time.

The author concludes that the partial thromboplastin time appears to offer the best control of anticoagulant therapy, but suggests that there may be some advantage in employing more than one form of test as a routine.

HISTOLOGY

A Thermoelectrically Cooled Microtome Table and Knife. Rutherford, T., Hardy, W. S. and Isherwood, P. A. (1964), *Stain Tech.*, **39**, 185.

This article describes the use of Frigistor Thermo-elements to replace CO_2 for microtome stage and knife cooling. The thermo-elements were used on Reichert and Leitz sledge microtomes. Leitz and Lipshaw freezing microtomes and the Cambridge rocking microtome. Fixed tissue can be cut using the stage cooling unit only by a knife cooling unit is required to cut unfixed tissue. Stagecooling is rapid, -36°C in 40-60 secs. and the temperature easily maintained. Serial sections were cut at 7μ.

D.T.

Sandwich Embedding of Eyeball Wall for Optimal Paraffin Sections of Retina. Sutter, E. and Meier-Ruge, W., (1965), *Stain Tech.*, **40**, 19.

Whole eyeballs are fixed in Susa, mercurial pigments are removed by 0.5% iodine in 80% alcohol, pieces of bulbar wall are enclosed between two pieces of formalin fixed liver (dehydrated to 80% alcohol) bound with thread and processed to paraffin. The thread is removed and the block embedded. The histological elements of the retina are well preserved. D.T.

A Cholinesterase-Bielschowsky Staining Method for Mammalian End Plates. Gwyn, D. G. and Meardman, V. (1965), *Stain Tech.*, **40**, 15.

Motor end plates, in 50μ frozen sections of muscle, are outlined using the acetylthiocholine iodide-copper sulphate technique to demonstrate cholinesterase. The sections are then further fixed for 15 days in 10% formalin containing 2% pyridine in saline, followed by a modified Gros-Bielschowsky stain to demonstrate the nerve fibres entering the motor end plates. D.T.

MICROBIOLOGY

A Vertical Diffusion Method for the Microbiological Assay of Isoniazid. Lloyd, Janet and Mitchison, D. A. (1964), *J. clin. Path.*, **17**, 622.

A method is described for the assay of isoniazid in serum and other body fluids, by diffusion along slopes of Lowenstein-Jensen medium inoculated with tubercle bacilli. The method is convenient, rapid and robust, but is less accurate than diffusion systems for the assay of some other substances.

Report on Antibiotic Sensitivity Test Trials Organised by the Bacteriology Committee of the Association of Clinical Pathologists. (1965), *J. clin. Path.*, **18**, 1.

As the result of the antibiotic sensitivity trial organised by a committee of experts certain recommendations have been made. Their findings which are related to work undertaken in 154 different laboratories highlight gross errors which may be associated with routine sensitivity testing. The value of uncontrolled tests is discussed and the committee supports the view held by clinicians that some reports are unreliable. This article will call for further work before the solution is found. H.C.W.S.

An Improved Lactose Gluconate Medium for the Detection of *Escherichia coli* and Other Coliform Organisms in Water. Gray, R. D. (1965), J. *Hyg. Camb.* **62**, 495.

Laboratory workers who are responsible for the examination of water supplies may wish to introduce this new medium into their laboratory. The author claims that the cost is less than a quarter of that of MacConkey broth and is at least as efficient for the routine testing of water samples. Furthermore, fewer false positive reactions occur. H.C.W.S.

Hair as a Reservoir for Staphylococci. Summers, Margaret, M. Lynch, P. F. and Black, T. (1965), *J. clin. Path.* **18**, 13.

The occurrence of *Staphylococcus aureus* in the hair of the scalp was investigated. The groups of people were examined, outpatients, in-patients, and staff of a general hospital. Bacteria were grown from the hair of all subjects tested. *Staphylococcus aureus* was the commonest pathogen isolated. It was more frequently found in the hair than the nose but 20% of hair carriers were not nasal carriers. There were more staphylococcal post-operative wound infections in hair carriers than non-carriers and in such cases the pathogen was the same phage type as that isolated preoperatively from the hair. The article has provided valuable information for cross-infection control. H.C.W.S.

PARASITOLOGY

Identification and Characterisation of Antigenic Components of Sheep Hydatid Fluid by Immunoelectrophoresis. Chordi A. and Kagan I. G. (1965), *J. Parasit.* **51**, 63.

Sheep hydatid fluid was examined by immunoelectrophoretic methods. On analysis nineteen antigenic components were found. Tests with homologous antisera showed that ten of the bands produced were of parasitic origin. In a group of diagnostic sera eight of the ten bands were identified. This advanced contribution may well be followed by a major modification of existing diagnostic methods. This article may well provide some support to the discussion made in a relevant article appearing in this issue of our own *Journal*. H.C.W.S.

SEROLOGY

Evaluation of a New Preserved Latex Antigen for the Sero-Diagnosis of Rheumatoid Arthritis. Goldin, M. and Black, A. (1964), *Ann. rheum. Dis.*, **23**, 485.

A new concentrated preserved latex antigen for use in quantitative testing for the rheumatoid factor is compared with two widely-used slide tests and the F II latex tube test. It is found to be satisfactory, specific and sensitive. The advantages of the new antigen are discussed.

The Health Department Examinations

INTERMEDIATE — MARCH 1965

Written Paper 1

Time allowed 2 hours

1. What methods of sterilisation are used in the laboratory? In each case, state briefly the basic principle involved and the equipment used.
2. Describe your method for the isolation and identification of the gram-negative intestinal pathogens.
3. Detail a method for the estimation of chlorides in a specimen of cerebro-spinal fluid.
   Outline the principle involved.
4. (a) By means of a table, show the interaction of cells and serum in the ABO groups.
   Write notes on:
   (b) The E.S.R.; (c) Eosinophils; (d) Reticulocytes.

Written Paper 2

Time allowed 2 hours

1. State the probable causes of the following troubles in the use of a binocular microscope. What steps would you take to trace the cause of the trouble, and to correct it?
   (a) A small dark spot, visible constantly in the same part of the field;
   (b) Inability to focus with the oil-immersion objective, although no difficulty is experienced with the other objectives;
   (c) When searching a stained smear, it keeps going out of focus;
   (d) One side of the field appears brighter than the other;
   (e) Eye-strain or difficulty in using both eyes together.
2. Describe the standard procedure for non-urgent cross-matching of blood for transfusion.
   Discuss the importance of correct identification and labelling of blood-specimens and tubes in cross-matching.
3. Suppose that you are on the laboratory staff of a newly-established hospital in a tropical region. It has been impossible to obtain supplies of commercially-produced modern aids to urinalysis, but equipment and chemicals are available for the traditional methods. Compose, in simple language, a set of precise instructions for the nurses who will be expected to do routine ward testing of urines for reaction, specific gravity, protein, sugar and acetone.. (Use diagrams where you think they would be helpful.)
4. Write short notes on:
   (a) Culture and identification of *Brucella abortus*;
   (b) Prevention of glycolysis;
   (c) Bacitracin;
   (d) How to keep a burette in good working order;
   (e) Poisoning by mercury and its compounds in the laboratory.

SUCCESSFUL CANDIDATES

Anderson, M.	Auckland	Bryce, Miss A.	Auckland
Anderson, Miss P. M.	Wellington	Bumstead, W. J.	Christchurch
August, Miss P.	Greymouth	Burrows, Miss E. M.	Christchurch
Bent, Miss M. J.	Tauranga	Carthew, P. L.	Palmerston N.
Boyack, Miss M.	Wellington	Chalmers, D. G.	Wellington
Brocas, Mrs J. G.	Hamilton	Christian, Mrs E. M.	Hamilton
Bryan, P. R.	Auckland	Cleverley, Miss Y. M.	Timaru
Bryant, D. J.	Auckland	Colebrook, Miss V. A.	Auckland

Coltman, Miss J. M.	Nelson
Coxhead, Miss P. M.	Auckland
Dawson, Miss R. R.	Auckland
Duncan, Miss K. Y.	Napier
Eccershall, Miss L.	Hamilton
Edwards, B. T.	Christchurch
Elliott, G. S.	New Plymouth
Elliott, J.	Wellington
Erceg, Miss F.	Auckland
Evison, Miss G. E.	Hamilton
Flack, M. R.	Hastings
George, Miss D. E.	Invercargill
Gerring, Miss M. D.	Hamilton
Gilmour-Wilson, Miss	Masterton
Glover, G.	Hamilton
Gould, Miss M. L.	Wanganui
Graham, E. J.	Christchurch
Grant, Miss K. McK.	Hamilton
Hadfield, Miss J. M.	Blenheim
Hankers, Miss B.	Wanganui
Hawkless, Mrs J. R.	Hamilton
Hooley, Miss P. K.	Auckland
Kerr, Miss C.	Auckland
Kerr, Miss J. M.	New Plymouth
Kettle, P. R.	Nelson
King, Miss M. J.	Hamilton
McConnell, D. S.	Christchurch
McQuinn, Miss M. E.	Auckland
Maddocks, Miss P. A.	Wellington
Martin, Miss F. S.	Hastings
Maslen, Miss J. M.	Christchurch
Mold, Miss M. E.	Palmerston N.
Monteath, Miss J. S.	Dunedin
Montgomery, J.	Auckland
Neallie, J. D.	Auckland
de Nicolo, P.	Nelson
Oxnam, Miss N. E.	Nelson
Rae, B. A.	Christchurch
Ricketts, Miss J.	Auckland
Rimmer, G. L.	Auckland
Robbie, Miss L. M.	Balclutha
Rutherford, Miss F. R.	Auckland
Sarfati, Mrs P. M.	Wellington
Southern, Miss A. L. R.	Wellington
Strutton, Miss C. J.	Auckland
Subritzky, M. G.	Auckland
Thomas, Mrs J. R.	Wellington
Tibbles, B.	Greymouth
Tracey, R. J.	Napier
Usherkoff, Miss M. F.	Auckland
Wallace, Miss P. J.	Timaru

There were 79 candidates for the examination, 67 of whom were successful.

FINAL — CERTIFICATE OF PROFICIENCY (APRIL-MAY, 1965)

Written Paper — Chemical Pathology

Time allowed, 3 hours

Instructions to candidates: All questions are of equal value and within any one question all major divisions are of equal value.

1. (a) You are asked to set up a quality control system in the Chemical Pathology section of your laboratory. Discuss the salient points of the system you would recommend.

   (b) Write brief notes of *five* of the following:—
   (i) The mode of action of anticoagulants; (ii) Flame photometry; (iii) The tests available for the measurement of acid secretion by the stomach; (iv) Serum protein electrophoresis; (v) Paper chromatography; (vi) The glucose tolerance test; (vii) Automation in analysis.

2. (a) Imagine you have just been appointed to a position in charge of a newly set-up hospital laboratory, and you are given the chance to buy eight books and five journals for the chemical pathology section. What would be your selection?

   (b) The following estimations require special precautions, either in the collection of the specimen or in its subsequent handling before analysis. What are these precautions and why are they necessary:—
   (i) Catechol amines in urine: (ii) Glucose in blood; (iii) Bilirubin in blood; (iv) Urobilinogen in urine; (v) CO_2 content of plasma; (vi) Protein-bound iodine in blood; (vii) pH of urine; (viii) Calcium in urine.

   (c) Most blood for analysis is taken by venepuncture, but capillary blood is also often used. What factors would in-

fluence your choice of specimen? What constituents are present in different concentrations in these two types of blood?

(d) How would you check the wavelength calibration of a spectrophotometer?

(e) What substances may cause a sample urine to darken on standing? In what clinical conditions are they present, and what tests would you apply to distinguish them?

3. (a) Describe in detail the preparation of exactly decinormal carbonate-free sodium hydroxide solution.

(b) Describe the dangers, if any, in the use of the following chemicals:—
(i) Mercury; (ii) Nessler's reagent; (iii) Potassium ferricyanide; (iv) Phenol; (v) Benzene; (vi) Hydrofluoric acid; (vii) Methylene chloride; (viii) Chromic acid cleaning solutions; (ix) Ether; (x) Potassium cyanide.

(c) Indicate whether the following statements are true or false. (One mark is given for each correct answer, and one mark taken off for each incorrect answer).
1. When diluting sulphuric acid, one should add water to the acid.
2. A 10% w/v solution contains 10 grams of the solute dissolved in one hundred ml. of solvent.
3. Lactose will reduce Benedict's solution.
4. Phenolphthalein changes colour over the pH range 5.8 - 7.2.
5. Concentrated hydrochloric acid is about 15N.
6. The serum calcium is usually decreased in tetany.
7. Biuret is the compound formed when copper combines with proteins.
8. The molecular weight of urea is 62.
9. The pH of a solution is a measure of its titratable acidity.
10. Ascitic fluid can be obtained from the knee joint.

Written Paper — Haematology and Blood Bank Serology
Time allowed, 3 hours
Instructions: Candidates to answer all questions.

1. Describe in detail the tests you would carry out on a patient suspected to have suffered a haemolytic transfusion reaction. You should confine yourself to tests designed to exclude serological incompatibility. (Descriptions of biochemical or bacteriological procedures are not required.)
Supposing that you have detected an incompatibility, brief an outline of the procedure you would follow to establish the specificity of the antibody.
2. Discuss the causes of false positive and false negative results in the Coombs' antiglobulin test. Describe the way in which you, personally, would control your test to ensure confidence in the results.
3. Briefly give the meaning of the following terms:—
(a) Incomplete antibody, (b) Chromosome, (c) 'Blocked' cells, (d) Non-secretor, (e) Dangerous universal donor, (f) Pappenheimer body, (g) Heinz body, (h) Auer's rod, (i) Doehle (or Amato) body, (j) Selenoid body.
4. What is mean by the term 'megaloblastic anaemia'?
Outline the abnormalities you would expect to be present in the blood film of a patient suffering from megaloblastic anaemia.

Discuss briefly laboratory tests which may be used in the differential diagnosis of megaloblastic anaemias.

5. An elderly woman presents with spontaneous massive bruising. Describe how you would proceed to investigate systematically, by laboratory means, the nature of the haemorrhagic state. Discuss briefly the uses and limitations of each test you mention.

Written Paper — Microbiology

Time allowed, 3 hours

Question 1 is compulsory. Answer any three of the remaining four questions.

1. Make a critical appraisal of the present day methods employed for the isolation and classification of *M. tuberculosis*. In doing so cover the best means of ensuring that atypical strains are isolated by the methods recommended.
2. Give details of the methods employed for the isolation and classification of Beta haemolytic streptococci.
3. State the means whereby *C. albicans* may be isolated and identified from a sputum specimen.
4. State how you would prepare a batch of Wassermann antigen.
5. Give details of the techniques employed in the isolation and identification of *Cl. welchii* from pus containing several types of bacteria.

Practical Paper — Chemical Pathology

Time allowed, 3 hours

All questions to be answered.

NOTE: No textbooks to be used but method sheets for the urea and creatinine methods are provided.
Describe briefly the technique used where applicable and show calculations.

1. Measure the total concentration of sodium and potassium in the urine 'A' and express in milliequivalent/24 hours specimen. Measure the concentration of chloride and the carbon dioxide content in the serum 'A' and express in milliequivalents per litre. The table provided for the flame photometer is calibrated in millequivalents per litre when the solutions are diluted 1 in 50. A standard NaCl solution is provided for estimating chloride by Schales & Schales method.
   A formula for use with the manometric Van Slyke is provided for estimating the carbon dioxide content.
2. (a) Perform a total bilirubin estimation on serum 'B' by Powell's method using control serum 'C' as a standard. The bilirubin concentration is marked on the bottle.
   (b) Perform a urea estimation by the phenate-hypochlorite technique on the serum 'B' and process the control serum 'C' in parallel. Comment on the significance of your control result.
3. (a) Estimate the diastase in urine 'B' by Wohlegemuth's method. Define and express results in terms of the Diastatic Index.
   (b) Urine 'B' is an aliquot of a 24 hours urine specimen, volume 1680 ml.
   The plasma creatinine is 0.8 mg./100 ml.
   Measure the urine creatinine by the method provided and calculate the creatinine clearance.

Practical Paper — Haematology and Blood Bank Serology

Time allowed, 3 hours

1. Using the techniques with which you are familiar, carry out crossmatching tests to determine which, if any, of the three donors X, Y and Z are suitable for transfusion to patient W. Describe the methods you have used in detail and record your results.
   This is a non-urgent request and you are informed that the patient and all three donors are group O Rh(D) positive.
   (Supplied: Serum and a 50% washed cell suspension from patient W; 50% washed red cell suspensions from donors X, Y and Z; antiglobulin reagent ready for use without further dilution. Ask for any other materials your choice of method may require.)
2. Determine the probable rhesus genotype of each of the four red cell samples provided (S, T, U and V). Record your results in detail, and if the interpretation of the probable genotype rests on the reaction with another antiserum, indicate the specificity of the serum with which you would wish to carry out a further test and state your interpretation in the event of both a positive and a negative result.
   It is not practicable to furnish each candidate with control cells for each antiserum; so state the controls you would consider necessary to ensure accurate results with each antiserum you have used.
   (Supplied: 5% washed red cell suspensions from patients S, T, U and V; saline reacting anti-C and anti-E, albumin-reacting anti-D and anti-c; inert (AB) serum; 20% bovine albumin solution).
3. Comment on the blood films M, N, O, P, Q and R.
   (The films are stained by the May Grunwald-Giemsa technique.)
4. Stain the two unfixed blood films K and L for iron, examine them microscopically and make any comments that occur to you resulting from your examination.
   (Supplied: absolute methanol; 2% potassium ferrocyanide; 2% hydrochloric acid and 0.1% safranin.)

Practical Paper — Microbiology

Time allowed, 3 hours day, 1 hour following morning.
All questions should be attempted.

1. Broth cultures A and B contain gram-negative bacilli isolated from an adult patient with diarrhoea.
   In addition to determining the motility of each strain, inoculate the appropriate media provided. Such cultures will be incubated for you overnight. Tomorrow morning, record results of biochemical tests, state the possible identity of the two organisms and proceed to classify the pathogen by slide agglutination tests with the sera available.
2. Identify the fungus provided on slide culture.
3. Stain the four slides 3A, 3B, 3C and 3D by Gram's method. Examine them microscopically and record your observations.
4. You are presented with a slightly turbid fluid which is thought may have been obtained from a cystic structure. Examine the fluid and identify the nature of the lesion.
5. The three sera: 5A, 5B and 5C have been inactivated. Screen them for the presence of Brucella antibodies. Complete the investigation by carrying out a standard tube agglutination test, the final result should be read tomorrow morning.

(Suspensions, tubes and other reagents are available on your bench).

SUCCESSFUL CANDIDATES

Bateman, K. J.	Lower Hutt	Kelman, Miss J. C.	Christchurch
Beggs, W. A.	Auckland	Lee, Miss A. J.	Invercargill
Bott, Miss G. R.	Auckland	Lockwood, B. McK.	Palmerston N.
Carman, Miss M. G.	Wellington	Lumsden, Miss M. R.	Christchurch
Clarke, K. R.	Auckland	McBride, Miss R. H.	Auckland
Courtenay, W. J.	Auckland	Marr, J.	Whangarei
Davy, N. C.	Auckland	Mitchell, M. A.	Rotorua
Deacon, A. G.	Nelson	Moffatt, P. N.	Wellington
Drew, Miss M. G.	Wanganui	Parnham, Mrs R.	Lower Hutt
Drummond, J. D.	Dunedin	Pitches, D. J.	Auckland
Ford, M. R.	Auckland	Reeves, Miss H. F.	Lower Hutt
Gamlin, Miss B.	Palmerston N.	Scott, Miss E. L.	Dunedin
Holland, Miss S.	Auckland	Sorensen, Mrs M.	Wellington
Horrocks, Miss D.	Auckland	Stewart, A. McD.	Dunedin
Horton, Miss J. F.	Dunedin	Weston, O. G.	Auckland
Johnston, E. M.	Auckland		

Forty-nine candidates sat the examination, two in Microbiology only.

Thirty-one obtained a pass, eleven obtained a partial pass and seven failed the examination.

Book Reviews

Immunology for Students of Medicine. J. H. Humphrey, M.D., B.Ch., F.R.S., and R. G. White, M.A., D.M., B.Ch. Second Edition. Blackwell Scientific Publications Ltd., Oxford, England. Price in U.K., 47s 6d.

In recent years immunological techniques have been used for an ever increasing number of problems in many different spheres of clinical medicine. The consequent demand for serological tests is associated with technical problems which demand a fundamental understanding of immunology, rather than being related to a lack of manual dexterity. The publication of this work provides a realistic bridge between the practical application of serological methods and an allied theoretical background. The reading of this manual should do much to inspire confidence in work undertaken both in the routine and applied research laboratory. If by popular demand a 3rd edition becomes necessary a change of title might well extend the range of appeal to those actively engaged in day to day routine serological work.

At a glance it is apparent that the book is roughly divided into two halves, chapters one to six will have a special meaning to the medical laboratory technologist, whereas the remainder of the work has a more clinical bias. Nevertheless, everyone should take advantage of the simple style of presentation, which immediately holds the reader's attention. In particular, the basic philosophy related to complement fixation and antibody-antigen reaction is very well presented. On the other hand, the reviewer is not really convinced that every unexplained abnormality neatly fits into the pattern of auto-immune response.

A second reading of the book illustrates the advantage of a well prepared glossary which caters for those perhaps less familiar with the new jargon. In all I would thoroughly recommend this classic amongst recently published medical literature. H.C.W.S.

Progress in Medical Laboratory Technique — 3. Ed. F. J. Baker, F.I.M.L.T., F.I.S.T., F.R.M.S. Butterworths, London, 1964. 250 pages.

The third volume in this series covers a comprehensive range of medical laboratory techniques, as did the previous two. A good deal of interesting information is presented in concise form, but little, if any,

of it is new. In fact, the exact value of the publication is open to some question. The author states in his forward, "The newer techniques being interspersed with information which, although not necessarily new, is nevertheless not easily found in standard text books." The reviewer would beg to differ. Many of the techniques mentioned are far from new, and any medium-sized laboratory would probably have had the techniques in use before this volume was published. The general review articles on sterilisation, microscopy and auto-immunity bring together, however, a good deal of current literature in readable form.

Some Aspects of Sterilisation (J. Dick): The review covers the physics of steam, steam supply, gravity displacement dressing steriliser, high vacuum dressing steriliser, and is an up-to-the-moment review of current sterilising procedures in use in major institutions. A useful section for trainees is on steam physics and sterilising controls.

Diagnostic Staining Procedures (R. Silverton): A summary of developments in staining over the past few years emphasises the value of the well-established methods.

Freeze-drying of Tissue by Thermoelectricity (J. Bancroft): This section has little practical application in a routine laboratory, but trainees will find useful information on the principles, purposes and practice of freeze-drying.

Some Applications of Fluorescence Microscopy in Histopathology (D. Munday): A review of established techniques, and also some useful information on fluorescence in general.

Recent Developments in Microscopical Techniques (J. Bassett): This review covers image conversions, staining, design trends, fluorescence microscopy, photomicrography and polarising microscopy. A very useful review ranging over many fields and succeeding very well in bringing the reader abreast of developments in microscope instrumentation.

Enzymology (J. Sinnott): Reviewed are international units of enzyme activity, serum creatinine, phosphokinase and serum alpha-hydroxy butyric dehydrogenase.

Some Applications of Thin Layer Chromatography (D. Kilshaw): Once again standard and well-tested techniques form the basis of the chapter. The methods for separation of carbohydrates do not find wide use, as better methods are available. This is particularly true in the separation of glucose from galactose, where Rf values of 0.17 and 0.18, respectively, are given. Similarly with barbiturate separation.

For any laboratory large enough to be doing chromatography, there is nothing new to be found here and, in fact, some recent techniques are not presented at all.

Auto-immunity in Certain Diseases (G. Ormsby): This gives a good introduction into the theory of an increasingly important field. Details of some techniques are accurately described, but there are occasional unfortunate omissions, such as a lack of detail on the Rose Waaler variants. While reference is made to the availability of tests detecting antinuclear antibodies for the diagnosis of D.L.E., the fluorescent antibody technique is the only one described. This is the least used in routine practice and is considerably more difficult to perform than the more specific latex and complement fixation tests, which were not even mentioned.

R.T.K.

Council Notes

A Council meeting was held at Wellington Hospital on May 22, 1965.

Present were Mr H. G. Bloore (in the Chair), Miss J. Mattingley and Messrs C. W. Cameron, J. Case, M. McL. Donnell, E. K. Fletcher, H. E. Hutchings, R. T. Kennedy, J. D. R. Morgan and D. J. Philip.

Applications and Resignations

Applications for membership approved:

Associates

Stewart, Miss H. G. Tauranga

Members

Abbott, Miss E. M. Dunedin
Alderton, K. J. Christchurch
Ames, Miss R. W. Christchurch
Anderson, A. P. Auckland
Anderson, R. K. Rotorua
Beattie, Miss M. J. Auckland
Bede, Miss S. A. Dunedin
Bedford, Miss D. M. Dunedin
Bent, Miss M. V. Auckland
Bibby, Miss E. J. Hamilton
Blumhardt, Miss R. Auckland
Bodger, B. J. Christchurch
Bowen, Miss B. E. Wellington
Briggs, Miss L. M. Auckland
Buchanan, Miss J. Auckland
Cardon, Miss P. Auckland
Christie, Miss H. N. Balclutha
Clark, Miss C. F. Auckland
Clarke, Miss N. P. M. Auckland
Cleave, Miss S. Auckland
Cleaver, Miss J. Wellington
Collins, R. Auckland
Cullens, Miss H. Christchurch
Cunningham, Miss M. M. Dunedin
Davidson, Miss P. J. Auckland
Dixon, M. W. Auckland
Dodd, G. W. Dunedin
Dohrman, D. Christchurch
Don, Miss B. J. Dunedin
Dumpleton, Miss D. Nelson
Forrester, Miss E. McC. Dunedin
Garrett, Miss D. M. Tauranga
Glenn-Killeen, Miss M. Auckland
Godsall, Miss J. I. Oamaru
Gould, Miss I. D. Hawera
Griffiths, R. F. Auckland
Harrison, Miss M. Christchurch
Hearn, Miss L. New Plymouth
Holland, Miss E. Christchurch
Hulse, Miss G. M. Auckland
Johnstone, Miss M. C. Dunedin
Jones, Miss P. Christchurch
Lane, Miss P. Christchurch
McCrae, Miss S. L. E. Auckland
McIver, R. K. Dunedin
Martin, Miss H. Napier
Martin, Miss J. Napier
Mason, Miss D. A. Dunedin
Mawson, Miss P. Hawera
Morris, Miss S. Christchurch
Mulvey, T. B. Auckland
Pape, Miss M. L. Hamilton
Parkinson, Miss S. S. Auckland
Reilly, Miss D. M. Hamilton
Richmond, W. J. Invercargill
Robinson, Miss A. M. Auckland
Roberts, M. W. Auckland
Samuels, Miss A. Waipukurau
Sargent, S. E. M. Hawera
Simms R. Palmerston North
Simich, Miss A. M. Auckland
Slack, Miss H. Auckland
Smith, Miss C. M. Auckland
Smith, Miss P. Christchurch
Spiers, R. Wellington
Still, Miss J. R. Hamilton
Stinear, Miss J. W. Auckland
Tanner, Miss K. Tauranga
Tong, Miss L. M. Hamilton
Walton, Miss V. F. Auckland
Watson, Miss M. G. Auckland
Webber, Miss A. Hamilton
Wesley, E. A. Auckland

Members elected as Associates

Allan, R. D. Dunedin
Buchanan, Miss A. M. Auckland
Cameron, C. W. Dunedin
Cathcart, D. Invercargill
Grace, A. I. Wellington
Kitto, J. B. Dunedin
Olsen, R. E. New Plymouth
Phillips, O. R. Auckland
Speden, Miss J. Christchurch
Tingle, D. Dunedin

Reinstated Members

Glover, G. Auckland
Hamilton, T. Auckland
Phillips, O. R. Auckland
Taylor, D. M. Otahuhu

Resignations accepted with regret:

Clark, K.	Auckland	Lawton, Miss D.	Hamilton
Douglass, Miss H. L.	Wellington	Peddie, J. J. G.	Upper Hutt
Healey, Miss R.	Auckland	Rhodes, Miss H. M.	Hamilton
King, Miss M. J.	Hamilton	Webb, Mrs R. M.	Whangarei

and to take effect at the end of 1965:

Sadler, Mrs G. Christchurch

Mr J. J. G. Peddie was a foundation member of the N.Z. Association of Bacteriologists, and the Secretary was directed to extend to him the best wishes of the Council for a long and happy retirement.

On failure to pay subscriptions for 1964/65 and in the absence of written resignation, the following names were removed from the Roll in accordance with Rule 10 (c):—

Alexander, Mrs M.	Christchurch	Irvine, Miss S. P. N.	Auckland
Bailey, Miss V. A.	Auckland	Johnston, N. D.	Kaitaia
Bathgate, P.	Auckland	Jones, Miss A.	Auckland
Beal, Miss J. G.	Hamilton	Joy, Miss P.	Auckland
Beech, M. J.	Auckland	McBride, Miss R.	Auckland
Burroughs, Miss B. J.	Auckland	McDowell, Miss H.	Rotorua
Burrows, Miss E.	Christchurch	McDuff, D. A.	Ashburton
Carr, Mrs M. I.	Wellington	McLoughlin, P.	Rotorua
Cornere, B. M.	Auckland	Martin, Miss B.	Auckland
Coulton, Miss D.	Greymouth	Neilson, Miss S.	Wellington
Cox, Miss M. H. F.	Christchurch	Nicholls, Miss J. M.	Hamilton
Curtis, C. S.	Auckland	Nixon, A. D.	Auckland
Dold, G. E.	Hamilton	Parrish, V.	Auckland
Elliott, J. E.	Wellington	Ricketts, Miss J.	Auckland
Ferguson, Miss D.	Whangarei	Robinson, Miss J. A.	Wellington
Forsyth, A.	Dunedin	Samuel, J. A.	Suva
Gardner, Miss G. K.	Wellington	Smith, Miss J.	Wellington
Gibson, W. B.	Christchurch	Steven, Miss S.	Auckland
Godkin, Mrs V. E.	Wellington	Ushakoff, Miss M.	Auckland
Gooch, Mrs M.	Tauranga	Watt, G. W.	Auckland
Grant, Miss K. M.	Hamilton	Weston, G.	Auckland
Grattan, M. J.	Christchurch	Wheelhouse, Miss J.	Auckland
Gray, Miss L. J.	Invercargill	Whitefield, Miss J. G.	Whangarei
Hampton, Mrs V.	Christchurch	Williams, Mrs D. G.	Auckland

New members enrolled during 1964 but who had failed to pay an initial subscription were also removed from the Roll:

Barnett-Smith, Miss K. A.	Auckland	Opie, C. A.	Wellington
Brown, P. B.	Wellington	Smythe, Miss P. N.	Christchurch
		Smythe, Miss R. H.	Wellington

In view of the number of new applicants who enjoy the privileges of membership for a year and then default on the subscription, the Council decided that in future new members should not be placed on the regular mailing list until they have paid their current subscriptions.

Honorary Membership

The roll of Honorary members was reviewed, and it was decided to recommend the Annual General Meeting to pass a resolution to restrict the number of Honorary Members to sixteen. Surviving Honorary Members at this date are:

Drs Cairns, Doyle, S. Hills, M. Fitchett, D. N. Allen, M. G. Somerville, T. H. Pullar, J. O. Mercer, P. P. Lynch, K. F. M. Uttley, D. T. Stewart, E. F. D'Ath, Sir C. E. Hercus, Mr S. Josland and Dr C. W. Taylor.

Public Service Investment Society

The Council were informed that the Secretary had at last received advice that members of the Institute employed at public hospitals could be afforded the discount service available to Public Service employees. The decision is provisional, but will be ratified in due course, and further details will be available soon.

Hospital Service Tribunal

It now seems that the months of negotiation towards the establishment of a Tribunal for hospital employees will eventually be satisfactorily concluded.

The President reported that in recent months substantial progress has been made. A questionnaire had been sent to fourteen organisations representing the interested groups of hospital workers, and eight organisations had given full approval for the negotiations to proceed These were the original five (N.Z. Dietetic Association, N.Z. Registered Occupational Therapists Association, N.Z. Physiotherapists Association, N.Z. Registered Nurses Association and the Society of Male Nurses of N.Z.) plus N.Z. Medical Physicists Association, N.Z. Dental Officers Association and our our own Institute. Of the remaining organisations, the Medical Superintendents Association and the Medical Specialists indicated that they would prefer to retain the present system; the Engineers, Radiographers and Orthopaedic Technicians wanted to give the matter further thought; and the Hospital Officers Association had not replied.

The Committee had now felt able to make a fresh approach to the Department of Health and, accordingly, a deputation representing the eight approving groups met the Minister of Health in May. The Minister was sympathetic and gave an undertaking that the submissions would be investigated. There is little likelihood that this will be possible during the present session of Parliament, but in the meantime the Minister asked for the Committee to prepare their proposals in fine detail, which is a task presently being undertaken. This will take several more meetings of the Committee, and if the final draft of the proposals are available in time, it may be possible for the President to introduce them for the information of the Annual General Meeting.

The loss of interest on the part of certain of the formerly interested groups need not be an obstacle to the establishment of a Tribunal. The Minister indicated that there was no reason why submissions from employee groups not wishing to be dealt with by the Tribunal should not continue to be heard by Salaries Advisory Committees.

The manner in which the Tribunal will be likely to work is, broadly, that the organisation wishing to be heard will outline their proposals to the Employers and ask that a date be set for conciliation. If an agreement is reached, its terms will be submitted to the Tribunal, which will give the force of law to the decision. Finalisation will, in most cases, take no longer than a few months, and in the case of the Public Service Tribunal there is no recorded instance in which the Tribunal has refused to ratify an agreement amicably reached by negotiation between employees and employers.

If agreement cannot be reached at the first level, the subject of the dispute will be referred to the Tribunal, which will appoint assessors to hear and consider the opposing points of view. The Tribunal will then make known its decision, generally within a month or two.

State Registration in Britain

The Institute has been asked to furnish details to enable the Medical Laboratory Technicians Board in London to consider the eligibility of the Certificate of Proficiency Examination as a suitable equivalent qualification for State Registration in the United Kingdom.

The information required includes copies of the syllabus; examination papers, for both Intermediate and Final examinations, both Theory and Practical, covering the last three years; the number of candidates examined at each centre; the names of the society or societies providing examiners; the percentage pass rate and information on the educational prerequisites for training and examination and the amount of practical experience called for during or after training.

This information is at present being compiled by the Secretary and will be completed when the results of the recent C.O.P. Examination have been published.

The matter of reciprocity of qualifications has long been a source of concern to the Institute, and the Council is hopeful that the situation of holders of the C.O.P. accepting positions in Britain may soon be bettered by a favourable decision on the part of the Board.

Annual Conference 1965

The Secretary reported to the Council that the cost of chartering aircraft to fly members to Tauranga from Wellington would be prohibitive. In view of the fact that there is only one scheduled DC 3 flight daily from Wellington, members would be well-advised to make their reservations early in order to leave plenty of time for the National Airways Corporation to provide a special flight if it should prove necessary.

Career Brochure

Biological Laboratories Ltd., of Auckland, have very kindly offered to subsidise the printing costs of the career brochure which it is the intention of the Council to prepare. The layout of the brochure is under consideration, and it is hoped that it can be made available before the end of this year.

The 1965 Examinations

The Council heard that the Department of Health had authorised Hospital Boards to reimburse candidates in the recent examination for their actual fares for travel to Dunedin.

Medical Laboratory Technologists Board

The President reported that the Higher Examination syllabuses were now drafted in full and were being considered. There had also been much work put in on amendments to the new syllabus, which would be printed in the revised form later this year.

The sub-committee on training had met and had considered the various ways by which training could be conducted through local educational establishments. The Central Institute of Technology would want to take over the training completely. Live lectures would be given at local polytechnics, and there would be correspondence tuition for those trainees unable to attend centres where lectures would be available. It would be necessary for correspondence students to attend the Central Institute for a one to three week period of practical training each year. Hostel accommodation would be available at a reasonable cost, and it was reasonable to expect that fares would be paid by employers.

Meanwhile, in Britain the findings of the Watford Report seem to suggest that it may be wise to follow some course other than that of attempting to continue training on a part-time basis.

Evening classes are considered, there, to be of limited value in teaching theoretical principles. Students are tired after a full day's work and are frequently not able to assimilate the material given in the lectures. Day release, too, creates problems, and the most practical solution is thought to be the abandonment of the present "apprenticeship" system and the introduction of "sandwich" courses, in which half of each year is spent in full time theoretical and practical training and half is spent in

a hospital laboratory. The Report advocates the establishment of special teaching establishments, with training laboratories entirely divorced from routine work, attached to base or teaching hospitals and staffed by people qualified to teach. The ideal aimed at is two or three-tiered qualifying systems, perhaps with a degree awarded by one of the new colleges of advanced technology as the highest qualification, with possibly a senior and junior technical diploma available for laboratory workers not willing or not able to reach this level.

The Council felt that it would be unwise for the Medical Laboratory Technologists Board to proceed without taking note of the fact that evidently the evening class system has proved inadequate to overseas. There was complete agreement among the Council that something along the lines of the suggestions made in the Watford Report should be considered. However, it is clear there has been no definite decision on future action in Britain as yet, and it would seem that the I.M.L.T. is hostile to any move that may result in the management of the examinations slipping farther from its control. The "sandwich courses" seem to offer the only means by which degrees in medical laboratory technology can become available, but there has already been comment in the British press about ways of distinguishing these degrees from "real" university degrees, perhaps by the use of an asterisk.

Free Meals for Laboratory Staff on Overtime

The availability of free meals for medical laboratory technologists who are unable to leave the hospital premises is something that seems to vary in different hospitals. Some hospitals allow meals on a grace and favour basis, while others expect their employees to pay, even though house surgeons and certain other hospital workers are entitled to receive meals without payment.

The Secretary was instructed to write to the Director-General of Health, pointing out the position and asking for consideration of what seems to be an injustice.

Regional Council Members

It was agreed that in the event of the transfer of a regionally elected Council member from one region to another by virtue of a change of employment, he should automatically resign from the Council in favour of a fresh nominee from the region being left unrepresented. No precise procedure for this was decided upon.

Back Numbers

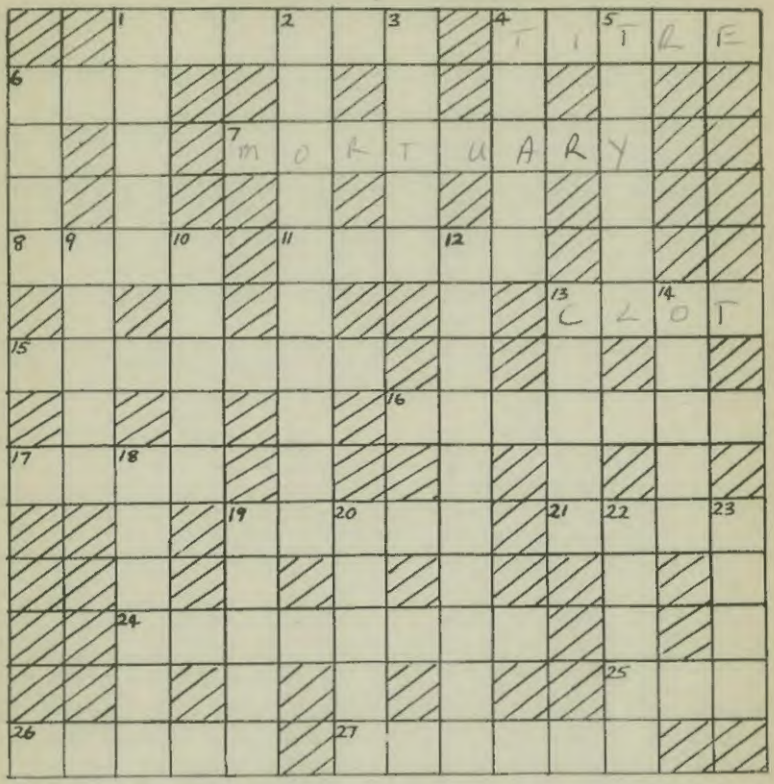
From time to time, inquiries are received from subscribers to the *Journal* for complete sets of back numbers, or for particular issues which are now out of print. A small number of copies of each issue is always kept in stock to meet such requests, but it has occasionally been necessary to supply subscribers with incomplete sets when stocks of certain issues become exhausted.

Numbers out of print, for which there have been requests, are the following:—

Volume 8, No. 1 (April 1953); Volume 9, No. 1 (April 1954); Volume 15, Nos 4 & 6 (April and October 1961) and Volume 17, No. 1 (April 1963).

If any members have copies of these issues which are no longer required, the Editor would be grateful to receive them, for which the proper rate of 2s 0d per copy will be paid.

Laboratory Crossword



Clues Across

1. Non-protein containing substance capable of antibody stimulus.
4. Reciprocal of the highest dilution at which an antibody will react with an antigen.
6. Collagen disease (abb.).
7. Dead centre of most hospitals.
8. Agar . . . on cooling.
11. Subject of the methylene blue reduction test.
13. 100 followed by a lot, you idiot.
15. Quantitative test for urinary proteins.
16. Seen under the microscope.
17. Creatine, creatinine, urea, etc.
19. Ego antibody.
21. Famous serologist in a hurry?
24. A legalish turn of dysentery?
25. Object.
26. I am in bran making thromboplastin.
27. Heterozygous state of congenital anaemia.

Clues Down

1. Centre of the vascular system.
2. A test for melanogen, with the Scandinavian god making a good start.
3. One of Africa's newborn states?
4. Body fluid containing lysozyme.
5. Used in a liver function test.
9. Used in the Romanowsky stains.
10. Pertaining to blood films and rather messy.

12. *Aerogenes* is one of these.
13. Not turbid.
14. Concerned with microscope systems.
18. Anticoagulated blood contains this.
19. The opposite of a ferrous cat.
20. Doctors may do this to patients.
21. Maps the top of the spine.
23. The drudge at the end of the course that is dreaded by all.

Solution on page 101.

Branch Reports

AUCKLAND

(Secretary: I. C. King, Pathology Department, Green Lane Hospital, Auckland.)

The following office-bearers were elected at the Annual General Meeting:—

Chairman:	Mr J. Walsh
Secretary:	Mr I. C. King
Treasurer:	Miss G. Walton
Committee:	Mr T. E. Miller
	Mr R. T. Kennedy
	Mr J. G. Meredith

CHRISTCHURCH

(Secretary: Mr E. Norman, Pearson Laboratory, 273 Montreal Street, Christchurch 1.)

There were nine meetings of the Branch during the past year with a notable decline in attendance over the last two meetings.

Among the guest speakers were Miss D. Bryant who spoke on her travels in Iceland; Dr D. T. Stewart, Director of Pathology, North Canterbury Hospital Board, who demonstrated the histological appearance of some common lesions with the aid of the xenon microprojector; a panel discussion on the origin, progress and objectives of the N.Z.I.M.L.T.; Mr Brooks who demonstrated some aspects of Medical Photography; Dr G. C. T. Burns and Mr R. C. Bridger answered some bacteriological and mycological queries; Dr P. Fitzgerald showed members over the Cytogenetics Unit and discussed the work of the Unit.

At the Annual General Meeting of the Branch it was decided to limit the number of formal meetings to three per annum and to supplement this with specialised discussion groups at members' homes, it being felt that interest would possibly be greater at smaller, more informal groups.

Officers elected for the current year are:—

Chairman:	Mr F. L. N. Corey
Secretary/Treasurer:	Mr E. Norman
Committee:	Mr G. R. Rose
	Mr T. E. Tanner
	Miss M. Eales

T.E.T.

DUNEDIN

(Secretary: A. McD. Stewart, Pathology Department, Medical School, Dunedin.)

Only one meeting has been held by the Branch this year: on April 7th. After a short business discussion, Mr E. Sutcliffe, B.Sc. from the Dominion Laboratories, addressed the meeting on various aspects of the work undertaken by the Government Analyst.

This year it was the Branch's responsibility to organise the South Island Seminar at Ashburton. Twenty-one members were present at the

Seminar, five of whom presented papers. The committee's thanks are due to the Ashburton Hospital Board for making available their facilities, and also to Mr J. Horner for his valuable assistance in helping with the organisation.

The next meeting of the Branch is in June to discuss possible remits for Conference.

A. McD. S.

WELLINGTON

(Secretary: Miss J. Cuthbert, Pathology Department, Wellington Hospital.)

Officers for 1965:—

Chairman:	Mr A. Grace
Secretary:	Miss J. Cuthbert
Committee:	Miss P. Anderson
	Mr B. McLean

Auckland Branch One-Day Seminar

On October 17, 1964, the Auckland Branch of the N.Z.I.M.L.T. held another of its one-day seminars at the Auckland Public Hospital. The day was fine and the attendance excellent, with 113 persons signing the roll. Representation came from Gisborne, New Plymouth, Ruakura Animal Research Station, Hamilton (private and public laboratories), Rotorua (both hospitals), Tauranga, Taumarunui, Whangarei, Thames and all private and public laboratories in Auckland.

A buffet tea and cocktail evening, attended by some 40 persons, elimaxed a most successful day.

The following is the programme presented to those attending:—

Opening of seminar by Dr S. E. Williams, Director of Laboratory Services.

Renal Physiology (Dr Taylor).

Laboratory Aspects and Management of Renal Disease (Dr R. Farrelly).

The Formed Elements in the Urine and their Significance (Mr T. Miller).

A Survey of Quantitative Bacterial Counts and the Bacteriology of Mid-stream Urine Specimens (Mr J. Holland).

Immunological Tests in Renal Disease (Mr A. Fischman).

Determination of Calcium by Flame Photometry (Mr J. Pybus).

Lathe Ruthven Bilirubin Method (Miss R. McBride).

An Investigation into Some Aspects of Staphylococcal Pathogenicity Tests (Miss S. Holland).

The Routine Examination of Synovial Fluid (Miss V. Drewitt).

Contagious Pustular Dermatitis (Mr W. Orbell).

Medical Mycology (Mr F. M. Rush-Munro).

The Tragedy of Errors (Mr P. Curtis).

The Hazards of Exposure to Mercury in a Medical Laboratory and in Industry (Mr B. W. Barry).

J.T.H.

One-Day Seminar

MANAWATU-HAWKES BAY-TARANAKI GROUP

A one-day seminar was held on Saturday, May 29, 1965, at the Palmerston North Hospital Medical Library.

Mr K. Archer, Surgical Superintendent, opened the seminar, welcoming sixty delegates and expressing pleasure, on behalf of the Palmerston North Hospital Board, in acting as host to medical laboratory technologist visitors.

Proceedings began with a forum on Microbiology. A presentation on antibiotic sensitivity testing and control drew much debate, while a paper on pregnancy testing proved very revealing, both for the number of methods available and the hazards which may be encountered.

This session was followed by lunch, then a browse through the Palmerston North Hospital laboratories. Many aspects of medical laboratory work were displayed in a most informative and interesting way.

The afternoon session began with a forum on Haematology. Once again the ever-valuable discussion on Blood Serology and crossmatching, also an unusual case history and a paper on unusual cell types. This forum ended with a lively debate on haemoglobinometers and haemoglobin standards.

The final session of the day was Biochemistry, which was perhaps noted for its informality of discussion and a most dramatic exit of the micro-Astrup (unrehearsed, of course).

The day closed with a vote of thanks on behalf of visitors to the Palmerston North Hospital Board; Mr K. Archer, Surgical Superintendent; and Mr H. E. Hutchings, Convenor of the seminar and able chairman of the day's proceedings.

Following are subjects presented and discussed:

MICROBIOLOGY:

1. *Fluorescent Microscopy—Direct and Indirect Labelling* (Mr K. Couchman, Palmerston North).
2. *Antibiotic Sensitivity Testing and Control—Routine and* M. tuberculosis (Mr T. Mann, Palmerston North).
3. *Use of Chemotherapeutic Substances in Microbiology—A Means of Typing and Classification* (Mr A. Harper, Wanganui).
4. *Pregnancy Testing—Commercial Kits and Animals—Reliability and Hazards, Disadvantages and Advantages* (Mr O. Jarrett, Palmerston North).
5. *Isolation of* Shigella sonnei *from Urine in a Case of Diabetes Mellitus and its Significance* (Mr G. D. C. Meads, New Plymouth).

HAEMATOLOGY:

1. *Cross Matching Techniques—Antibodies—Panagglutination—Rouleaux* (Mr H. E. Hutchings, Palmerston North).
2. *A Haematological Phenomenon—Lymphocytic Phagocytosis* (Mr G. Pearman, Hastings).
3. *Various Haematological Staining Techniques — Analysis — Illustrated with Slides* (Mr A. Williams, Palmerston North).
4. *Megalocytic Anaemias—Laboratory Diagnosis on Peripheral Film* (Dr Saunders, Palmerston North).

BIOCHEMISTRY:

1. *Blood pH, PCO_2 and PO_2—the Astrup—Theoretical and Practical* (Mr L. Margolin, Palmerston North).
2. *Reporting Electrophoretic Patterns—Comparative—Using Standard Normal Curve* (Mr D. Fisher, Hawera).
3. *Lactate Dehydrogenase—Total and Heat Stable Fraction—Simple Routine Estimation* (Mr E. K. Fletcher, New Plymouth).
4. *Iodoacetate—a Preservative—in Blood Glucose when Estimated by the Enzyme Technique* (Mr G. S. Elliott, New Plymouth).

E.K.F.

South Island Seminar

The South Island Seminar was held this year on Saturday, March 20, in the Nurses' Tutorial Block at Ashburton Public Hospital.

The Seminar was attended by 58 technologists from Christchurch, Ashburton, Timaru, Oamaru, Dunedin and Invercargill laboratories.

Mr B. W. Main, Chairman of the Dunedin Branch, welcomed members on behalf of the Ashburton Hospital Board, as the Surgeon-Superintendent was unable to be present.

The programme was divided into three sections: Haematology, Microbiology and Chemical Pathology, which were chaired by Miss M. Eales, Mr H. C. W. Shott and Mr R. D. Allan, respectively.

Papers presented were:

The Euglobulin Lysis Test. Miss M. Eales.
A Slotted Card System and its Uses. Mr T. E. Tanner.
Section Cutting of Sputa as an Aid to Diagnosis in Cytology. Mr B. W. Main.
Food Poisoning and the Isolation of Causative Organisms, Especially Clostridia. Miss R. Rusbatch.
Colicine Typing of Shigella sonne. Mr H. C. W. Shott.
The Intravenous Glucose Tolerance Test. Miss D. Bryant.
Automation in Analysis. Mr J. Braidwood.
Enzymes, Methodology, Units and Normals. Mr C. Cameron.

Mr L. Taylor introduced a discussion on pregnancy tests. Mr J. D. R. Morgan, Hon. Secretary, N.Z.I.M.L.T. (Inc.), gave a brief summary of current Institute affairs, and the Editor of the *Journal*, Mr J. Case, appealed for more material for the *Journal*.

The Ashburton Hospital Board kindly supplied lunch and morning and afternoon tea. Members had an opportunity to inspect the trades displays supplied by Watson Victor Ltd., Townson & Mercer Ltd., and G. W. Wilton and Co. Ltd., during the day.

A dinner and social function was held in the Somerset Hotel in the evening to conclude the day's activities. A. McD. S.

The Library

List and Contents of New Periodicals Received

Librarian: D. S. Ford, Pathology Department, Medical School, Dunedin.

Amer. J. med. Technol. Volume 31, No. 2 March-April, 1965.

Contents: Statistical Evaluation of Student Technical Performance; Blood Grouping Tests—Application to Related Scientific Fields; Multiple Concentrations of Constituents in a Quality Control Program; Rapid Antibacterial Sensitivity Testing Using A Tetrazolium Disk Technique; Dissemination of Bacteria by Laboratory Personnel; Blood Chlorides — Many Problems, Some Solutions and a Few Recommendations; Is Quality Control a Reality in Blood Banking? Quality Control has its Brightest Day in Clinical Chemistry; Quality Control in the Clinical Laboratory; What Can Quality Control do for Hematology? Contamination of Vaginal and Cervical Smears by Alternaria.

Ann. Med. exp. Biol. Fenn. Volume 42, No. 4. 1964.

Selected contents: Gas Chromatographic Identification of Pregnanediol and some of its Isomers in Bile of Pregnant Women; Significance of Hassall's Corpuscles in the Light of their Morphological and Histochemical Appearance.

Volume 42, No. 3. 1964.

Selected contents: Polyhydric alcohols in Human Urine; Variation of Blood Sugar in Finnish Twins; Studies on the Quality of Neutralizing Bacteriophage Antibodies Produced by Single Cells.

Volume 42, Supp. 3. 1964.

Contents: Immunological and Biological Properties of Exopthalmos-Producing Substance.

Aust. J. biol. Sci. Volume 18, No. 1. February 1965.
Volume 18, No. 2. April 1965.

Canad. J. med. Technol. Volume 26, No. 6. December 1964.

Contents: A Physiological Study of Platelets; The Laboratory Investigation of Three Cases of Paroxysmal Cold Haemoglobinuria; A Comparison and Evaluation of Colorimetric Procedures for 3-Methoxy-4-Hydroxymandelic Acid.

Volume 27, No. 1. February 1965.

Contents: The Use of Proteolytic Enzymes; Les Anaerobies en Bacteriologie Medicale; Organizing a Cytology Service to Accomodate a Population Screening Programme; A Simplified Leukocyte Technique for the Study of Human Chromosomes; Problems in the Assay of Coagulation Factors.

Filter. Volume 36, No. 3. September 1964.

Contents: An Evaluation of a Rapid Kit Test for the Estimation of Blood Cholinestrase Activity; An Exhibit Laboratory.

Volume 36, No. 4. December 1964.

Contents: The Detection and Identification of Atypical Antibodies Encountered During Crossmatches in Hospitals in Southern California; The Catalase Test at 68°C.; The Coagulation of Blood; Laboratory Procedures in Medical Mycology.

Volume 37, No. 1. March 1965.

Contents: Dermatitis in Laboratory Personnel Due to *Bdellonyssus bacoti* (Rat Mite); Eluates—A Tool in A/O Diagnosis; Notes on the Interpretation of Treponemal Tests for Syphilis; Resources for Treponemal Tests for Syphilis in California; An Agglutination Test for the Differentiation of Leukemoid States from Leukemia.

J. Med. Lab. Technol. Volume 22, No. 1. January 1965.

Contents: Evaluation of a Pyruvate Medium in the Routine Isolation of Mycobacteria from Sputum; Recent Modifications to our Technique of Preparing Thin, Flat, Polished Tooth Sections; A Method for Rapid Distinction between Beta-haemolytic Group D Streptococci and Beta-haemolytic Streptococci of Other Lancefield Groups; The Detection of Thyroid Antibodies by Immunoelectrophoresis; An Automatic Cuvette Emptying Device for Unicam Absorptiometers; A Slow-growing Strain of *Pseudomonas pyocyanea*.

Lab. Management. Volume 3, No. 1. January 1965.

Selected contents: Planning to Get the Most out of Spectrophotometry; Modernizing Your Laboratory Skills; Housing the Laboratory Animal; What Kind of a Laboratory Environment is Needed for Maximum Creativity? Toward Safer Transfusion; Forensic Laboratory Service.

Lab World. Volume 16, Nos. 1, 2, 3. January, February, March, 1965.

Med. Surg. (Baroda) Volume 4, Nos. 11 and 12. November, December, 1964.

Volume 5, Nos. 1, 2. January, February, 1965.

Med. Technol. Aust. Volume 6, No. 3. July 1964.

Contents: Toxoplasmosis; A Review of the Importance of Serum Glutamic Oxalacetic Transaminase Estimations; Some of the Physical and Physiological Aspects of Diving.

Volume 6, No. 4. October 1964.

Contents: An Historical Survey of the Institute; an Historical Review of the Examining Council of N.S.W. 1938-64; The Examining Council in Veterinary Laboratory Technology N.S.W.

Volume 7, No. 1. January 1965.

Contents: Demonstration of Neurosecretary Material in the Hypothalmus and Posterior Pituitary, with Special Reference to a Case of Diabetes Insipidus; The Use of Dispersol in Culture Media for the Isolation of Streptococci; International Congress of Medical Technologists; Toxoplasmosis Part 2.

Microbiologia (Buc.) Volume 9, No. 6. November-December 1964.

Selected contents: New Data on the Pathogenesis of Infections with *B. anthrax* and Anti-anthrax Immunity; the Laboratory Diagnosis of Infections Caused by *Clostridium perfringens;* Parainfluenza Viruses; Epidemic of Streptococcal Angina of Alimentary Origin*; A Clinical Case of Ornithosis, Typhoid-like Form, in Man*; Food Poisoning with *Salmonella typhimurium* following the Consumption of Cottage Cheese*; A method for the Isolation of Germs Belonging to the Genera *Shigella* and *Salmonella* from Polluted Waters and Faeces when they Are Present in Very Small Number; Modification of the Cystinase Test Used in The Bacteriologic Diagnosis of Diphtheria; On the Cystinase Test Used in Slanetz Selective Medium Used for the Isolation of Enterococci*.

All articles in Rumanian. *English summary.

New Istanbul Contr. clin. Sci. Volume 7, No. 3. July 1964.

Contents: Studies on Urinary Excretion on Delta-Aminolevulinic Acid in Cases of Saturnism and in Plumb Workers; A Method for the Study of Factors Maintaining Uric Acid in Supersaturated Solution in Urine; Excretion of Electrolytes and Water during Osmotic Diuresis in Normals and in Patients with Chronic Renal Insufficiency; The Acid-Base balance of Arterial Blood and of Cerebrospinal Fluid with Reference to the Ventilation Status of Psychotic Patients; Mast Cell Leukemia with Bone Lesions; Simple Plastic Apparatus for Two Chamber Technique of Paper Chromatography.

N.Z. Hospital. Volume 17, Nos. 3 and 4. January, March, 1965.

Offic. J. Amer. med. Technol. Volume 26, No. 6. November-December 1964.

Contents: The Pattern of Cancer Research in 1964; Fluorometric Analysis in the Clinical Laboratory; Some Problems of Methodology; Clinical Estimation of Urea Nitrogen in Biologic Fluids by a Modified Nesslerization Technic; Clinical Estimation of Urea Nitrogen in Biologic Fluids by a Modified Bertholet Reaction; Serum Haptoglobin-A Screening Technique; New Tests from Laboratory Suppliers; A method for Differentiating the Colonies of Staph, Strep and Pneumococci on Blood Agar.

Volume 27, No. 1. January-February 1965.

Contents: Quality Control in the Laboratory; Sparganosis in Man; Salicylates in Biologic Fluids; Bromides in Biologic Fluids; Techniques—Laboratory Tips—From Readers.

Rev. viernes Med. Volume 15, No. 3 December 1964.

S. Afr. J. med. Lab. Technol. Volume 10, No. 4. December 1964.

Contents: Serum Uric Acid Estimation; Serum Creatinine Concentration Using an Ion Exchange Resin.

Tonic. Volume 2, No. 6. January 1965.

Volume 3, No. 1. 1965.

What's New

STAINLESS STEEL PRODUCTS

The Stainless Steel Fabricators' Association of Great Britain publishes a list of its members, which also includes a classified list of stainless steel products. If anyone is interested in receiving a copy of the list, the Secretary of the Association has indicated willingness to send one. The address of the Association is: Chamber of Commerce House, P.O. Box 360, 75 Harborne Road, Edgbaston, Birmingham 15, England.

ACCURATE CHOLESTEROL ESTIMATIONS ON JAUNDICED SERA

A new product of the Warner-Chilcott organisation is a non-reactive aluminium hydroxide adsorbent known as Seramox, which eliminates bilirubin interference in cholesterol assays.

Seramox is being marketed in bottles of 125 capsules, with 120 mg. of the bilirubin adsorbent in each capsule, at 40s 0d per bottle.
Further details from: *Wm. R. Warner & Co. Ltd., P.O. Box 430, Auckland.*

URASTRAT—A Simple New Urea Nitrogen Assay System

Urastrat is a quantitative urea nitrogen assay system which is said to be rapid, simple and accurate. A single test takes about a minute of working time, a 10 mm. x75mm. test tube and a *Urastrat* column — although the procedure is actually a built in series of complicated steps.

The reagents are premeasured and banded on the *Urastrat* column.

Low on the strip is a band of high-potency buffered urease, developed after earlier trial and error with ordinary urease, which proved to be insufficiently stable. (Over a period of time, minute traces of chemical substrates reacted with the urease and with other enzymes present to produce ammonia. Since *Urastrat* was designed to measure ammonia, the contamination resulted in falsely high readings; but, after experiments with special dialysis techniques, a urease was developed from which the traces of ammonia-producing substrates had been removed.)

Above the urease is a band of potassium carbonate; above this a narrow plastic barrier; and above this again the indicator band, bromcresol-green plus tartaric acid.

The performance of the test is simplicity itself:

The *Urastrat* column is placed in a test tube containing 0.2 ml. of serum or plasma. As this is drawn by capillary action up through the paper, it passes through a series of chemical adventures.

When it encounters the urease at the bottom level, the urea in the sample is converted to ammonia. As it migrates upward, the ammonia comes into contact with the potassium carbonate at the next level, and is liberated as a free gas. The plastic barrier prevents passage of the liquid sample, but the liberated gas builds up in the test tube, passes the plastic barrier and reacts with the tartaric acid in the indicator band, to produce ammonium tartarate. The resulting change of pH causes the indicator to change from yellow to blue-green, in a height directly proportional to the amount of ammonia in the sample.

The interpretation of the result is achieved by measuring the height of the colour change, which can be done by means of a special caliper of which a small number are available for issue gratis to laboratories purchasing *Urastrat*.
Inquiries to Wm. R. Warner & Co. Ltd., P.O. Box 430, Auckland.

INCREASING VERSATILITY OF THE AUTOANALYSER

Among new automated laboratory procedures reported to be possible on the *AutoAnalyzer* is the counting of red and white blood cells.

Using the new cell counter module, counts are possible without

predilution of the sample, and resulting from the complete automation of the procedure, precision is increased.

The New Zealand agents for the *AutoAnalyzer* are *E. C. Lackland & Co. Ltd., P.O. Box 5814, Auckland.*

A NEW AID FOR SERUM OR PLASMA SEPARATION

A dramatic new aid for blood separation is offered by a new product known as *Sep-ar-aid.*

Manufactured by the Unitech Chemical Manufacturing Company of the United States and shortly to be marketed in New Zealand by Biolabs of Auckland (subject to the granting of an import licence), *Sep-ar-aid* is an inert substance which, when added to a clotted or anticoagulated blood sample, forms a barrier between the clot or cells and the liquid phase of the blood.

Recovery of plasma or serum is simplified by the fact that the centrifuging time can be reduced to two minutes, and it is not necessary to pipette off because the interlocking of the particles makes it possible to decant without fear of disturbing the cells.

For further details, write to: Biological Laboratories Ltd., Private Bag, Northcote.

A PORTABLE BUNSEN BURNER

Newly on the market is a means of enabling a bunsen burner to be used in a room or laboratory not equipped with a mains gas supply.

The *Gas-Pak* bunsen burner is a cheap, efficient and small portable gas burner, that comes with a supply of odourless butane gas in disposable, self-sealing pressure cans. The gas cans are no larger than the well-known aerosol cans containing household deodorants and insect sprays, and each one lasts for four hours of use.

The burners are available at 5ls 0d each, and the cans of gas at 9s 3d each, with special prices for quantity.

Further details can be obtained from George W. Wilton & Co. Ltd., P.O. Box 367, Wellington; or P.O. Box 1980, Auckland.

DISC ELECTROPHORESIS. A New Development in the Diagnosis of Disease

Using the apparatus manufactured by *Canalco* of Bethesda, in three sizes capable, respectively, of running one, six and 12-36 tests at a time, a method is available for the investigation, diagnosis and even forecasting of many disease conditions.

The analysis of serum proteins, serum muco-proteins, serum isozymes, and urine proteins and hormones is possible by disc electrophoresis. Separation is remarkably sharp and precise, and resolution is thousands of times more sensitive than paper, agar, cellulose acetate or starch block, and many times finer than starch gel; and is fast (30 minutes) and reproducible.

Write for further information and catalogue material to: George W. Wilton & Co. Ltd., P.O. Box 367, Wellington; or P.O. Box 1980, Auckland.

ACCURATE PROTHROMBIN TIMES ON AGED BLOOD SAMPLES

A unique new blood coagulation reagent, designed for the control of anticoagulant therapy, has been developed by the General Diagnostic Division of Warner Chilcott Laboratories.

Called *Simplastin A*, the new product permits testing of plasma samples that are over four hours old. It also allows for Factor VII testing without preparation of special substrates. *Simplastin A* is a lyophilized thromboplastin - calcium extract with Factor V and fibrinogen added. The addition of Factor V, which is highly unstable in normal plasma, eliminates the need for immediate testing of plasma samples and

allows the accurate estimation of prothrombin on samples mailed to the laboratory, thereby saving patients on anticoagulant therapy the inconvenience of frequent attendances at the laboratory.

Simplastin A is expected to cost the same as *Simplastin* (50s 0d per box of 10 vials, 20-determination size), and a trial shipment is expected to arrive in New Zealand during June.

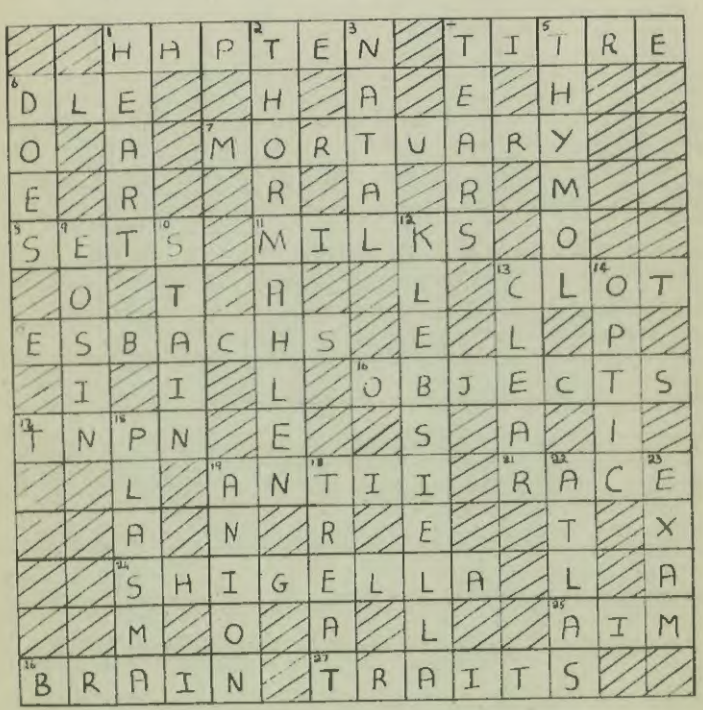
(Details obtainable from: Wm. R. Warner & Co. Ltd., P.O. Box 430, Auckland.)

NEW QUALITY CONTROL SERA

Chemonitor I and *Chemonitor II* are new quality control sera by Dade, with assayed values of human blood constituents for clinical chemistry determinations performed most frequently. *Chemonitor I* provides accurate known values for approximately thirty constituents in the normal range and *Chemonitor II* provides assayed values for approximately twenty-five constituents in the abnormal range.

Since no selective dialysis, extraction or deionization steps are used in the manufacture, these control sera react in the same way as test sera in all procedures, and, in the dried state, are stable for up to two years at 2° - 10°C.

(Details from George W. Wilton & Co. Ltd., P.O. Box 367, Wellington, or P.O. Box 1980, Auckland.)



Vacancies

Royal Perth Hospitol
Western Australia
Department of Haematology

MEDICAL LABORATORY TECHNOLOGISTS

Applications are invited from qualified Medical Laboratory Technologists to fill posts at this Hospital.

Royal Perth Hospital is the main teaching hospital associated with the University of Western Australia. The actual number of available beds utilizing emergency bed positions is 850.

The Department of Haematology is a large, modern, well-equipped one with facilities for specialised as well as routine investigations. The successful applicant will be required to undertake overtime and Call Duty as and when required, for which appropriate out of hours duty rates are payable.

QUALIFICATIONS: Candidates should have had a wide experience of haematological techniques and be an Associate of the Australasian Institute of Medical Laboratory Technology with the Diploma of Haematology and Blood transfusion techniques or equivalent qualifications.

SALARY: Commencing rate will be determined within the range of —

Male: £A1,466 - £A1,964 p.a.
Female: £A1,263 - £A1,761 p.a.

according to qualifications and experience. Other conditions of service include three weeks' Annual Leave, Sick Leave, Long Service Leave and Superannuation.

TRANSPORT EXPENSES: A travel allowance will be provided for the successful appointee and family (including children up to 16 years of age) from New Zealand, together with an allowance of up to £S200 (or £S25 in the case of a single person) for the removal of personal effects (excluding furniture and motor vehicle), subject to a bond to remain in the Hospital's service for a period of not less than three years.

Applications should state age, marital status, qualifications, experience, present post held, the names and addresses of two professional referees and should include a recent photograph.

Joseph Griffith,
Administrator

BACTERIOLOGIST

The Biochemistry Department of Lincoln College has a vacancy for a person with training and experience in bacteriology.

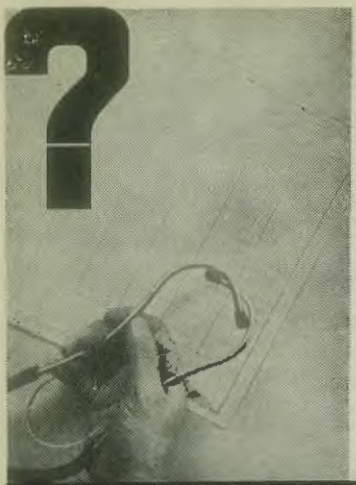
The appointee to this newly-created post will take charge of the isolation, cultivation and identification of the bacteria of the sheep rumen, as part of the Department's programme of research into the biochemistry of these micro-organisms.

The work will require a person with some years' experience in bacteriological laboratory practice, and preferably holding the Certificate of Proficiency in hospital laboratory practice.

Salary within the range £955 to £1,215 according to qualifications and experience, plus bus fares from Christchurch to Lincoln. Government superannuation available.

Conditions of appointment available from the undersigned, with whom applications close on July 15, 1965.

H. G. Hunt,
Registrar,
Lincoln College,
Canterbury.



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for urinary, respiratory, surgical, wound, burn and blood stream infections

- primarily bactericidal against most gram-negative pathogens — especially Pseudomonas and **E. coli** (not recommended for Proteus)
- exceptionally safe when used as recommended (exercise caution in presence of renal impairment)
- rarely induces bacterial resistance
- therapeutic blood and urine levels rapidly attained

Please remember: Since Coly-Mycin (colistin) is a polypeptide antibiotic, a clear zone of inhibition, regardless of size, indicates sensitivity — usually high sensitivity.

Sensitivity discs are available from your regular suppliers, from this office—free of charge, or your Warner-Chilcott representative. Also, have you seen the 4½ minute bacteriology film on Coly-Mycin (colistin)? Ask your Warner-Chilcott representative about it the next time he calls.

Side Effects: Occasional reactions such as circumoral paresthesias, nausea, dermatitis, drug fever, transient vertigo, and dizziness have been reported and usually disappear upon discontinuance of drug or reduction of dosage.

Precautions: Exercise caution in renal impairment. Transient elevations of BUN have been reported. As a routine precaution blood studies should be made during prolonged therapy.

William R. WARNER and Co. Ltd
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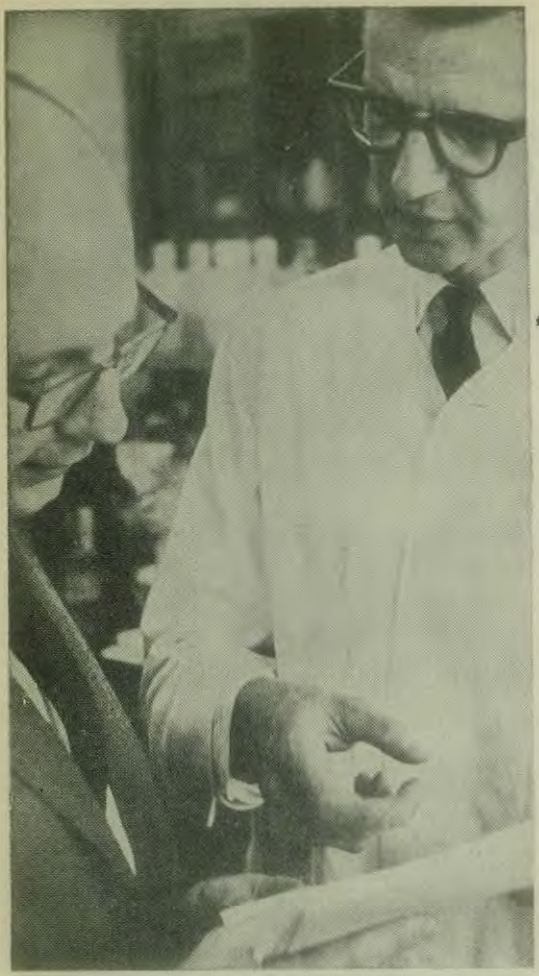

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Laboratory heads everywhere are answering clinicians with greater assurance, thanks to modern methods and materials for routine, daily quality control. The Versatol series, currently available comprise:-

Versatol: normal reference standard for 12 serum constituents

Versatol-A: abnormal reference standard for 16 constituents.

Versatol-A Alternate: alternate abnormal reference standard for 16 constituents.

Versatol Paediatric: reference standard for infant serum; normal for 13 constituents, abnormal for bilirubin (20mg./100ml.)

Details of the Versatol series, or information concerning the full range of Warner-Chilcott diagnostic reagents is available from the N.Z. agents on request:

William R. WARNER & Co. Ltd.

P.O. Box 430 Auckland

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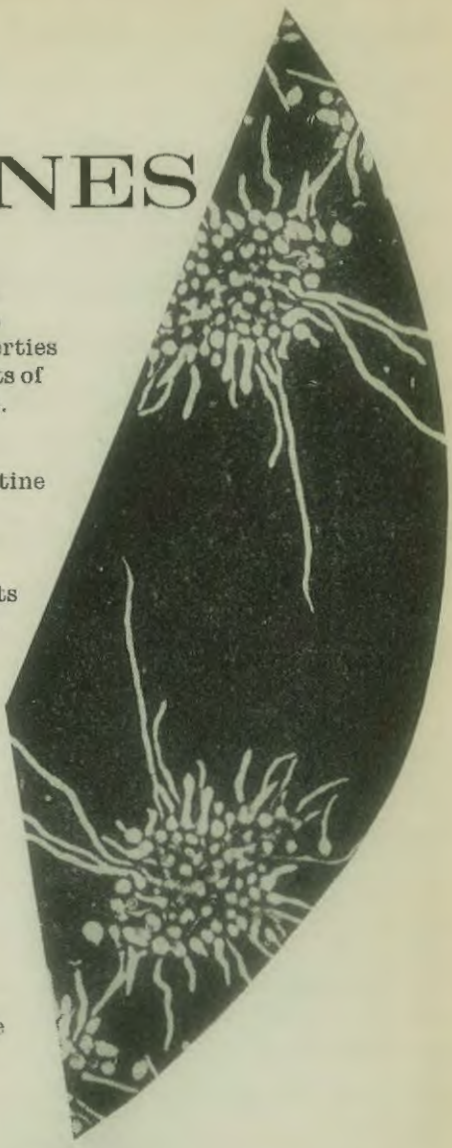
OXOID PEPTONES



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is used extensively for all routine purposes in laboratories throughout the world. It is a high-quality peptone which gives consistently good results with standard culture media.

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