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JOURNAL

OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

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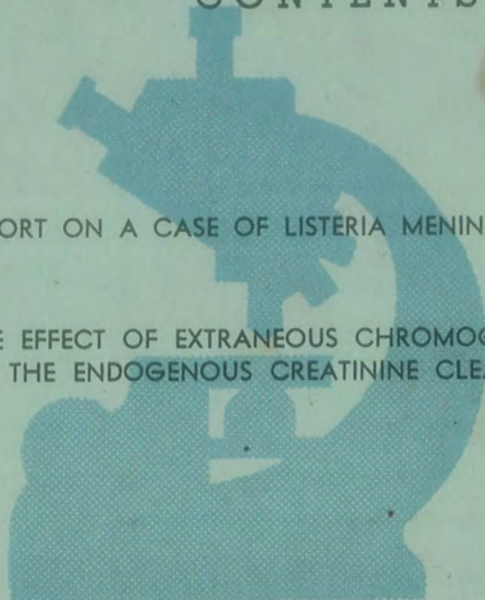

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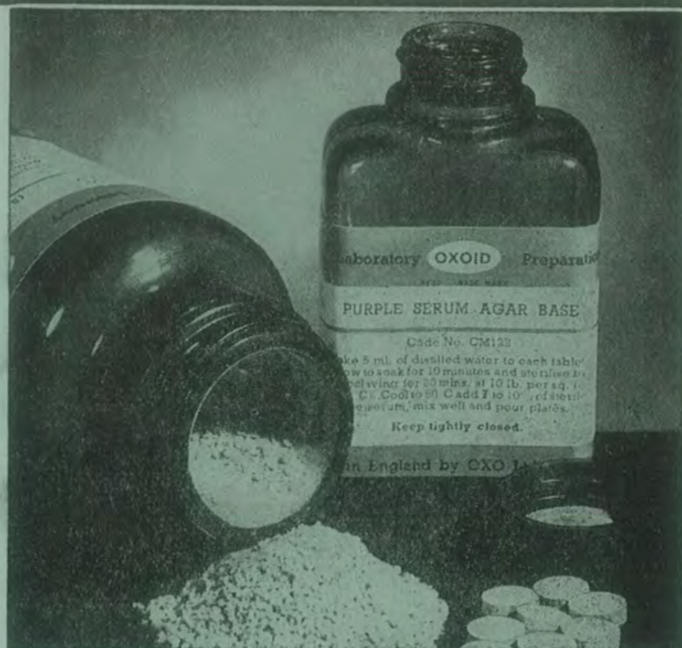

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REPORT ON A CASE OF LISTERIA MENINGITIS

J. Holland

(*Central Laboratory, Auckland Hospital*)

Listeriosis, an infection due to Listeria monocytogenes was first reported in England by Murray, Webb and Swann (1926). They described a disease of young rabbits and occasionally guinea pigs which had as one of its outstanding characteristics a well marked mononuclear leucocytosis. In smears of peritoneal fluid and infected organs a few small Gram positive bacilli were seen which on culture and subsequent intravenous inoculation of rabbits gave rise to an infection similar to the natural disease. The main characteristics of this organism, Gram staining, motility, failure to form spores and optimum temperature of 37°C were sufficient to exclude it from any recognised genus. The authors regarded it as a new species and, because of its effect on the large mononuclears, chose the name Bacterium monocytogenes.

Pirie (1927) in South Africa described an organism which caused a fatal disease among gerbilles. In natural infection the main lesions which Pirie noted were areas of focal necrosis in the liver. The name suggested by Pirie for the infecting organism was Listerella hepatolytica. This organism was compared by Murray et al with their own strains of Bacterium monocytogenes and was found to be identical.

In New Zealand, Gill, (1931) unaware of the work of Murray et al and Pirie, reported the presence of a small Gram positive organism in the lesions found by histological examination of the mid-brain of sheep suffering from an encephalomyelitis. The disease was known locally as "circling disease" because of one of the more obvious symptoms shown. In a subsequent article, Gill (1933) reported the isolation and a description of the cultural characters, pathogenic properties and relationship of this organism to the disease under investigation. The possibility of the parasite Oestrus ovis acting as a vector was also noted together with the observation that the biological and cultural characteristics did not allow classification of the organism in any of the recognised bacterial genera. He subsequently had no hesitation in placing it in the recently constituted genus Listerella.

Infection in humans was first recognised by Nyfeldt (1932) who isolated the organism from patients with an infectious mononucleosis syndrome. A total of 70 cases had been reported from various places by 1949. In Germany, Seeliger (1955) reported over 200 cases of listeriosis in the years 1950 to 1955.

The generic name Listerella and the species name monocytogenes are those given for Bacterium monocytogenes of Murray et al and Listerella hepatolytica of Pirie in the 4th edition of Bergey's "Manual of Derteminative Bacteriology". Topley and Wilson's "Principles of Bacteriology and Immunity" 3rd edition classifies the organism in the genus Erysipelothrix and points out the invalidity of the generic name Listerella, since it was given to a mycetozoan by Jahn in 1906. The 7th edition of Bergey's Manual gives the generic name Listeria in place of Listerella, thus overcoming the main objection to the use of the latter name. Infection due to Listeria monocytogenes is now termed Listeriosis and not Listerellosis as was formally the case.

The manifestations of listeriosis are predominantly meningitis and include granulomatosis of the foetus and newborn infant, conjunctivitis, endocarditis, urethritis and a type of infectious mononucleosis.

Two cases of neonatal listeriosis are described by Olding and Philipson (1960) in which the infection was probably acquired in utero. There were no clinical or pathological macroscopic signs to indicate an infection, but the histological and bacteriological investigations revealed massive infection in both cases.

The following is a report on a case of listeriosis in a female child aged 12 days admitted to the Princess Mary Hospital on 17th September 1959. Five days before admission the child had developed a cold with nasal discharge. On the day of admission the child appeared pale and grey, rolling her eyes and had a temperature of 102°. Lumbar puncture was performed with the following results:— R.B.C. 675,000/c.m.m, W.B.C. 1,800/c.m.m., polymorphonuclears 95%, lymphocytes 5%, protein 310 mgms%, sugar 40 mgms%, supernatant fluid colourless. Results of the examination of a second spinal fluid obtained the following day were as follows:— R.B.C. 180,000/c.m.m., W.B.C. 2,250/c.m.m., polymorphonuclears 70%, lymphocytes 30%, protein 392 mgms%. No organisms were seen in stained smears of either specimen. Cultures of both C.S.F. specimens and a blood culture resulted in the growth of a small Gram positive rod, sensitive by the disc method to penicillin, streptomycin, chloramphenicol, erythromycin and chlortetracycline. The child was treated with chloramphenicol until the 24th October and discharged well on the 7th November 1959. The final diagonis was septicaemia, acute otitis media meningitis.

BACTERIOLOGY:

The colonial appearances of the organism after 24 hours incubation at 37°C on nutrient agar containing 5% human blood

were similar to those of Streptococcus faecalis but the colonies were surrounded by a narrow band of beta-haemolysis. Gram stains revealed small non-sporing Gram positive rods 0.5 x 1.0—2.0 μ with pallisade formation a noticeable feature. No noticeable smell was detected. Motility was observed by swarming in 0.5% agar and hanging drop preparations of 4 hour broth cultures. The latter method demonstrated the tumbling motility more marked at 22°C than at 37°C which is characteristic of L. monocytogenes.

The following reactions were obtained in peptone water media containing 1% of the carbohydrate

Lactose—acid 72 hours
Glucose—acid 24 hours
Sucrose—acid 6 days
Maltose—acid 24 hours
Trehalose—acid 24 hours
Salicin—acid 24 hours

No change was observed in the following carbohydrate media during the 14 days incubation.

Mannite, Starch, Xylose, Arabinose.

Further tests were as follows:—

H_2S production negative, Indole negative,
Methyl red positive, Voges Proskauer positive,
Nitrate reduction negative, Gelatin liquefaction negative,
Catalase positive.

Growth was obtained on the following media:—

Anderson's tellurite agar, MacConkey agar, 6% NaCl broth.

Growth was also evident after 7 days at 4°C.

The organism was resistant to heat at 60° for 30 minutes but was killed after 60 minutes exposure.

A rabbit was inoculated intravenously with 0.5 ml. of an 18 hour broth culture. Death occurred in five days, the most prominent feature on post mortem examination being extensive necrosis of the liver. The organism was recovered in pure culture from the liver and pleural cavity.

A culture was forwarded to Dr. H. Seeliger who confirmed the identification, L. monocytogenes, and determined the serotype 4b.

SUMMARY:

A brief outline of listeriosis is presented together with the recognition 30 years ago of the disease in sheep in this country. A report on a case of human listeriosis is presented with the important bacteriological findings.

ACKNOWLEDGMENTS:

I would like to thank Dr. D. R. Goodfellow, Medical Superintendent, for permission to use the case notes presented and also thank Dr. H. Seeliger of Bonn University who confirmed the identification of the organism and determined the serotype.

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THE EFFECT OF EXTRANEOUS CHROMOGEN ON THE ENDOGENOUS CREATININE CLEARANCE

T. E. Miller
(*Chemical Pathology Department, Central Laboratory, Auckland Hospital.*)

INTRODUCTION:

In 1886 Jaffe published his paper describing the reaction of creatinine with alkaline picrate. Since then the estimation of creatinine has been of considerable interest to clinical chemists.

The specificity of the Jaffe reaction however has been questioned. Jaffe himself mentioned that acetone interfered with the reaction. Several other compounds of biological importance including glucose, protein, amino acids, ketone bodies, barbiturates, and ascorbic acid, have been shown to react as "non creatinine chromogens." (Taussky, 1956).

Methods for increasing the specificity of the Jaffe reaction towards creatinine have developed along four lines.

1. The destruction of interfering substances with ceric sulphate.

Kostir and Rabek (1950), Kostir and Sonka (1952), considered that the treatment with ceric sulphate removed pyruvic acid from the serum filtrate, thus reducing the total chromogen present by half.

2. Determination of creatinine using N.C. bacteria.

This method was used by Miller and Dubos (1937), who prepared a specific enzyme capable of decomposing creatinine while leaving the other chromogens intact.

3. Adsorption using Fullers earth.

This was first introduced by Gaebler and Keltch (1928), and used by Borsook (1935), Hare (1950), Haugen and Blegen (1953), Owen et al (1954), and Edwards and Whyte (1958). Their claim that Fuller's earth quantitatively adsorbs creatinine and thereby separates creatinine from interfering chromogens has been widely but not universally accepted. Furthermore the eluate was found to behave like a simple solution of creatinine.

4. Preliminary treatment of serum with iodine, zinc in acid solution, and finally extraction with ether.

This technique was introduced by Taussky and Kurzmann (1954), and Taussky (1956). They found that 51 substances of biological significance did not interfere when this technique was used.

Miller and Dubos (1937), estimated extraneous chromogen in serum after destruction of the creatinine with an enzyme and

found it to be responsible for 10% of the total colour in normals. However in a later paper (Miller and Miller, 1951), using both the enzymatic and adsorption methods claimed that 20% of the total chromogen was extraneous. Hare and Hare (1949), confirmed these findings.

Our interest was centred around the question of extraneous chromogen and its effect on the endogenous creatinine clearance test. As the serum creatinine level is the denominator in the clearance formula, the clearance figure will be lowered in proportion to the amount of extraneous chromogen present in the serum provided there is no chromogen in urine. Several investigations have compared serum levels of creatinine as determined by different methods but apparently only one (Roscoe 1958), has compared creatinine clearances.

MATERIALS AND METHODS:

(a) **Estimation of true creatinine in serum:**

Materials—

1. Sulphuric acid, 0.67 N.
2. Sodium tungstate, a 6% solution W/V in water.
3. Fuller's earth, B.D.H. (Lloyd's reagent, hydrated aluminium silicate). Each batch should be tested for satifactory adsorption.
4. Glass spoon measure to contain 100 mg. of Fullers earth.
5. Hydrochloric acid, 1.0 N.
6. Sodium hydroxide, 0.75 N.
7. Picric acid, a saturated solution in water. (Keep in a dark bottle).
8. Creatinine solutions.
   Stock standard. 250 mg. of pure creatinine or 405 mg. of creatinine zinc chloride dissolved in 250 ml. of 0.1 N hydrochloric acid. (Keep in refrigerator).
   Working standard. 5 ml. of the stock standard and 10 ml. of 0.1 N HC1 are made up to one litre with water. (This solution is stable at room temperature).
9. Spectrophotometer. A Beckman model DU was used in this investigation.

Method. (After Edwards and Whyte 1958).

If the plasma protein concentration is lower than normal, additional steps which will be described later must be taken.

Pipette 2.5 ml. of serum into a 6in. x 1in. pyrex boiling tube, 2.5 ml. of distilled water, 2.5 ml. of 6% sodium tungstate. Mix

well and add 2.5 ml. of 0.67 N sulphuric acid. Shake well, mix contents several times in 10 minutes. Centrifuge for 10 minutes at 2,000 r.p.m. and then filter through a small Whatman No. 1 paper. The filtrate should be crystal clear.

If the serum creatinine level is known to be higher than 6 mg./100 ml. dilute the filtrate at this stage by adding 2 ml. of filtrate to 10 ml. of distilled water.

100 mg. of Fuller's earth is placed in a glass stoppered centrifuge tube (glass stoppered tubes are essential) and 5ml. of the protein free filtrate added. This is followed by one ml. of 1.0 N. hydrochloric acid. Duplicate standard solutions and blanks containing 5 ml. of the working standard and 5 ml. of distilled water respectively are prepared similarly.

Mix several times in 5 minutes, centrifuge for 10 minutes at 2,500 r.p.m. and remove the creatinine free supernatent using a fine pasteur pipette. The test can be interrupted at this stage, if necessary, for several hours.

Freshly prepare alkaline picrate colour reagent, (10 ml. of saturated picric acid and 20 ml. of 0.75 N NaOH, made up to 100 ml. with distilled water.) To the button of Fuller's earth in each test tube add 5 ml. of colour reagent. Replace the stopper and resuspend the material by shaking several times in 5 minutes. Centrifuge for 10 minutes at 2,500 r.p.m. Decant the alkaline picrate solution into 5 x ⅝ins. test tubes and stand in the water bath at 25°C.

Read the optical density in a suitable spectrophotometer at wavelength 490 m.u. using distilled water as a reference. Readings must be taken as soon as the solution is removed from the bath before the temperature of the alkaline picrate changes.

When using this technique there are two practical points of considerable importance:

1. pH and protein precipitation.

   Various procedures have been reported to give different values for the recovery of added creatinine (Owen et al 1954). The procedures may be divided into two groups according to the pH of the serum filtrate:

   (a) Low titratable acidity $pH > 2.5$

   (b) High titratable acidity $pH < 2.5$

Recovery rates are minimal (80-95%) with filtrates having a pH of about 3.5 and quantitative when the pH is below 2.0. Since Folin and Wu (1919), published their original method for precipitation of blood proteins several authors, (Wu 1922, Brod and Sirota, 1948), have tried to improve on this method. Their

object was to obtain a low amount of extraneous chromogen in the serum filtrate.

Haugen (1953), has compared some of the modifications and concludes that the method of Brod and Sirota is to be preferred as this technique gives 100% recovery of creatinine and low values for extraneous chromogen.

With the advent of the Fuller's earth technique the question of pH and protein precipitants was still pertinent as protein that is incompletely removed shows a faint turbidity in the serum filtrate. It is absorbed together with creatinine on to Fuller's earth, and then acts as a chomogen. It is obvious that the protein content of the serum should determine the optimum concentration of the precipitants. In our investigations we first determined the total protein content of the serum and then made adjustments in the quantities of the other reagents. (Table I after Lauson, 1951).

TABLE I

TOTAL SERUM PROTEIN Gm. %	Volumes (mls.)			
	Serum	Distilled water	6% sodium tungstate	0.67 N H_2SO_4
5.8 - 8.0	2.5	2.5	2.5	2.5
4.5 - 5.8	2.5	3.5	2.0	2.0
3.0 - 4.5	2.5	4.5	1.5	1.5

2. The Effect of temperature on the alkaline picrate colour.

It has been pointed out by various investigators that variations in the temperature of the medium will effect the determination of creatinine. An increase in temperature will cause an increase in the optical densities of alkaline picrate and alkaline creatinine picrate. This effect is reversible. Therefore the readings should be made immediately after placing the solutions in the spectrophotometer. As the optical density of the reagent blank increases when the solution is repeatedly exposed to the heating effect of the light beam of the spectrophotometer (Haugen, 1953), water should be used as a reference optical density. In order that readings may be compared throughout the year we used a water bath maintained at 25°C in which the tubes containing the alkaline creatinine picrate stood before reading in the spectrophotometer.

Calculation:

$$\frac{\text{O.D. Test} - \text{O.D. reagent blank}}{\text{O.D. Std.} - \text{O.D. reagent blank}} \times 2 = \text{mg. true creatinine/100 ml.}$$

(b) **Estimation of total chromogens in serum.**

Materials:

1. 0.04 M. Picric acid—Prepare by taking 50 ml. of saturated picric acid solution and diluting to 100 ml. with water.
2. 0.75 N NaOH.
3. 0.8 N H_2SO_4.
4. Stock creatinine solution prepared as described under the method for "True Creatinine."
5. Working creatinine standard solution—4 ml. of the above stock solution are treated with 10 ml. of N/10 HC1 and the volume made up to one litre with water.

Method. (After Bonsnes and Taussky, 1945).

Pipette 2.0 ml. of serum into a 6in. x 1in. test tube. Add 5.5 ml. of water and 1.0 ml. of 10% sodium tungstate. Mix well and add 1.5 ml. of 0.8H H_2SO_4. Stand for 10 minutes and then centrifuge the mixture at 2,500 R.P.M. for 10 minutes . Remove the supernatent fluid. Pipette 5 ml. of the supernatant fluid into a 6in. x 1in. test tube and add 2.0 ml. of 0.04 M. picric acid. Mix and then add 2.0 ml. of 0.75 N NaOH. Prepare a reagent blank and standard by adding the picric acid and NaOH to 5.0 mls. of water and 5.0 mls. of working standard respectively. Read the optical densities in the spectrophotometer at 515 mμ *exactly* 20 minutes after the addition of the 0.75 N NaOH.

Calculation:

$$\frac{\text{OD test} - \text{OD reagent blank}}{\text{OD Std.} - \text{OD reagent blank}} \times 2 = \text{Total chromogens/100 ml.}$$

When this technique is used it is extremely important to measure the optical density exactly 20 minutes after the addition of the sodium hydroxide. Several investigations have mentioned that while a solution of pure creatinine will reach a maximum after which it is stable for several hours, a protein free filtrate under the same circumstances will continue to increase slowly for a variable period of up to 100 minutes before fading.

(c) **Determination of creatinine in urine.**

Fuller's earth is rarely necessary in this estimation. Dilute the urine 0.5 ml. to 100 ml. with water . Pipette 4.0 ml. of the dilute urine into a 6in. x 1in. test tube. Prepare a blank and standard using 4.0 ml. of distilled water and 4.0 ml. of the creatinine standard solution (0.5 mg./100 ml.). To each tube add 1.0 ml. of alkaline picrate solution prepared by adding equal parts of 0.75 N NaOH and saturated picric acid. Mix well and place in the waterbath at 25°C for 45 minutes. Measure optical densities at 490 m/μ.

Calculation:

$$\frac{\text{OD test} - \text{Blank} \times 100}{\text{OD standard} - \text{Blank}} = \text{mg. of creatinine/100 ml.}$$

Endogenous creatinine clearance.

24 hour urine samples were collected without preservative. A plain blood sample was collected at 1.0 p.m. on the day of the clearance. The endogenous creatinine clearance was calculated from the formula:

$$c = \frac{UV}{P} \times \frac{1.73}{SA}$$

c = clearance in ml./min.
U = urine concentration of creatinine in mg./100 ml.
V = volume of urine in ml./min.
SA = the patient's surface area in square metres calculated from height and weight by Dubois' nomogram.
P = Serum creatinine in mg./100 ml.

Normal values.

In normal ambulant males the serum creatinine concentration was 0.76 - 1.03 mg./100 ml. and in male hospital patients with no evidence of renal disease the concentration was 0.43 - 1.03 mg./100 ml. The corresponding endogenous creatinine clearances were 118 - 148 ml./min. and 100 - 120 ml./min.

In ambulant healthy females the serum creatinine concentration was 0.80 - 0.99 mg./100 ml. and for hospitalized patients with no evidence of renal disease 0.62 - 0.99 mg./100 ml. The creatinine clearance was from 87 - 123 ml./min. There was no significant difference between the creatinine clearance of the ambulant and hospitalized females.

RESULTS:

Table II shows the true creatinine, total chromogen, and extraneous chromogen concentrations in 50 sera from 37 patients. The extraneous chromogen varied from 0 to 0.19 mg./100 ml., and the per cent extraneous chromogen from 0 to 27.0%. The average chromogen content in the 50 sera examined was 8.7 per cent.

TABLE II

Sub j.	Sex	Surface Area (sq.m)	True Creatinine (mg/100ml)	Total Chromogens (mg/100ml)	Extraneous Chromogens (mg/100ml)	% Extraneous Chromogens	Creatinine Clearance (corrected)		Urinary Creatinine (mgs/day)
							Total Chromogens	True Creatinine	
1.	M	1.31	0.95	1.03	0.08	7.8	62	67	690
2.	F	1.51	0.78	0.81	0.03	3.7	102	106	1,040
3.	F	1.43	0.69	0.72	0.03	4.2	105	110	900
4.	F	1.58	0.87	0.85	0.00	0.0	65	64	735
5.	M	2.14	2.24	2.24	0.00	0.0	27	27	1,080
6.	F	1.73	0.93	0.90	0.00	0.0	83	78	1,030
7.	M	1.76	1.12	1.25	0.13	10.4	90	101	1,650
8.	M	1.76	1.27	1.27	0.00	0.00	61	61	1,130
9.	F	1.6	0.83	0.91	0.08	8.8	98	108	1,220
10.	M	1.67	0.88	0.95	0.07	7.4	105	114	1,390
11.	M	1.50	0.51	0.70	0.19	27.0	87	119	760
12.	M	1.50	0.68	0.79	0.11	14.0	109	127	1,070
13.	M	1.73	1.03	1.14	0.11	9.6	92	102	1,510
14.	M	1.63	0.55	0.70	0.15	21.4	67	86	635
1A.	M	1.31	0.84	0.97	0.13	13.4	54	62	555
16.	M	2.04	1.79	1.73	0.00	0.0	69	68	2,020
17.	M	1.98	1.37	1.39	0.02	1.4	91	92	2,060
18.	F	1.53	0.62	0.64	0.02	3.1	98	101	805
19.	F	1.53	0.90	0.85	0.00	0.0	64	61	700
20.	M	1.72	0.87	0.92	0.05	5.4	106	112	1,390
21.	F	1.51	0.76	0.85	0.09	10.6	59	66	630
22.	M	1.87	1.06	1.05	0.00	0.00	118	117	1,915
11A.	M	1.50	0.64	0.75	0.11	14.7	86	101	805
24.	M	1.73	1.02	1.15	0.13	11.3	90	102	1,490
14A.	M	1.63	0.54	0.64	0.10	15.7	71	84	730
3A.	F	1.43	0.67	0.78	0.11	14.1	95	111	890
27.	M	1.76	1.16	1.27	0.11	8.7	73	81	1,360
28.	M	1.80	1.08	1.12	0.04	3.6	106	110	1,770
29.	F	1.57	0.77	0.82	0.05	6.1	122	131	1,310
30.	F	1.64	0.90	1.01	0.11	10.9	37	41	510
31.	M	1.50	0.43	0.59	0.16	27.0	106	144	800
32.	M	2.02	0.94	1.01	0.07	7.0	111	119	1,890
33.	M	1.65	0.87	1.06	0.19	18.0	23	28	333
34.	M	1.77	1.31	1.31	0.00	0.00	90	90	1,720
22A.	M	1.87	1.05	1.10	0.05	4.5	127	133	2,160
7A.	M	1.76	1.09	1.17	0.08	6.8	102	110	1,760
37.	F	1.65	0.83	0.85	0.02	2.3	143	147	1,660
21A.	F	1.51	1.02	1.10	0.08	7.3	78	85	1,080
39.	F	1.60	0.75	0.87	0.12	13.8	82	91	950
40.	F	1.53	0.67	0.80	0.13	16.3	86	102	870
19A	F	1.53	0.93	0.99	0.06	6.0	61	64	765
42.	M	1.88	1.09	1.15	0.06	5.2	67	72	1,212
43.	M	2.02	1.47	1.61	0.14	8.7	21	23	560
37A.	F	1.65	0.86	1.05	0.19	18.1	101	123	1,440
30A.	F	1.64	0.87	0.99	0.12	12.1	82	97	1,110
43A.	M	2.02	1.66	1.69	0.03	1.8	82	84	2,350
42A.	M	1.88	1.14	1.17	0.03	2.6	76	78	1,400
48.	M	1.83	1.62	2.06	0.44	21.0	54	68	1,680
5A.	M	2.14	1.82	1.91	0.09	4.7	30	32	1,030
50.	F	1.49	0.65	0 77	0.12	15.6	58	69	550

Table II — True creatinine and total chromogen in serum. Determination of creatinine clearance using the figures obtained from determining true creatinine and total chromogen.

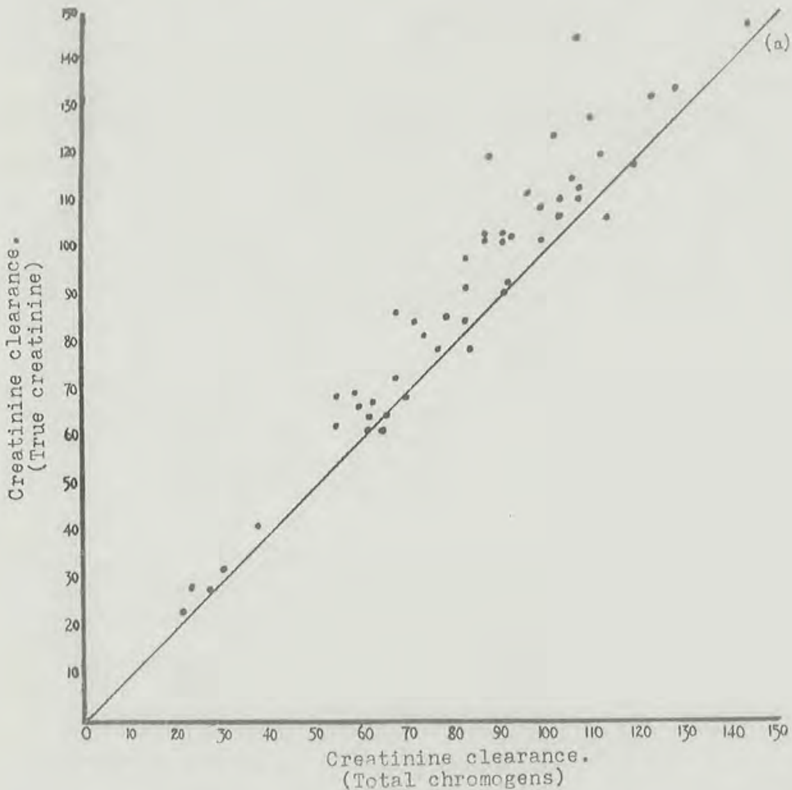

Fig 1. Comparison of the true creatinine clearance plotted against the creatinine clearance determined from the total chromogen content of the sera.

Fig. I illustrates the effect of extraneous chromogen on the endogenous creatinine clearance. The true creatinine clearance has been plotted as ordinates against the total chromogen creatinine clearance as abscissae. If the two techniques for estimating creatinine clearance were similar the points would lie along the line (a). The scatter shows the extent to which the creatinine clearance is increased by the estimation of the true creatinine figure. The points plotted in Fig I are taken from Table II.

DISCUSSION:

The results of previous investigators have generally agreed that approximately 20% of the chromogen in normal sera is extraneous chromogen. We have found that extraneous chromogen accounts for approximately 9% of the total chromogen measured. It has been mentioned that the interval between the addition of the NaOH to the picric acid filtrate and the recording of the optical density is of prime importance when determining total chromogen. Under the conditions of our test we found that a pure creatinine solution developed its maximum colour after 20 minutes. In our estimations of total chromogen this time interval was strictly observed. Had we read the optical densities of our solutions after 25 or 30 minutes as some authors recommended our extraneous chromogen figure would have been considerably higher.

The true endogenous creatinine clearance has been found to give results comparable with the inulin clearance, and has been recommended as an alternative to the inulin clearance. Whether a laboratory should use the true creatinine or total chromogen technique depends on the circumstances. Our results indicate that although the total chromogen clearance gives results significantly lower than the true creatinine clearance it is still a good index of glomerular filtration rate and suitable for daily clinical work.

However the technique for estimating true creatinine does not entail a great deal of extra work and this technique could readily be used in laboratories which estimate creatinine at irregular intervals. It should certainly be used in preference to the total chromogen clearance in scientific and research work.

SUMMARY:

The total chromogen present in serum filtrates and estimated by the Jaffe reaction is compared with the true serum creatinine estimated after adsorption on to Fuller's earth and subsequent elution. Practical points in the estimation of true serum creatinine and total chromogens are discussed. The non creatinine chromogen has been found to contribute 8.7% of the total chromogen colour. The effect of extraneous chromogen on the endogenous creatinine clearance has been demonstrated and the conclusion reached that while total chromogen clearance is satisfactory for routine clinical work the true serum creatinine clearance should be used for scientific and research work.

ACKNOWLEDGEMENT:

The author is indebted to Dr. R. O. Farrelly for help and advice during this study.

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LETTER TO THE EDITOR

The Editor,

N.Z. Institute of Medical Laboratory Technology,

Dept. of Pathology,

Public Hospital, CHRISTCHURCH.

Sir,

I have recently received a circular from the National Health Institute headed "Examinations for Medical Laboratory Technicians 1962."

Now Sir, we have recently brought our association into line with similar organizations overseas by adopting the title of the N.Z. Institute of Medical Laboratory Technology — could we not right from the outset take a stand against this term "Technician"? Why should our association not define the terms, perhaps as follows?

MEDICAL TECHNOLOGIST: Any person employed in medical laboratory work and who possesses the C.O.P. qualification or equivalent.

LABORATORY TECHNICIAN: Any person employed in a laboratory who does not possess the C.O.P. or equivalent qualification and who is employed in non-professional work such as glassware preparation or media manufacture.

With official definitions adopted at least we would know just where we stand. We rightly insist on a high standard of training and ethics. Let us insist on the correct title.

R. W. BARRINGTON.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY (INC.)

Minutes of Council Meeting held at New Plymouth on June 14th, 1961.

The meeting opened at 4.35 p.m.

Those present — Mr Olive in the chair, Messrs Philip, Cameron, Donnell, Bloore, Walker and Misses Scarf, Mattingly and Evans.

Mr Mead by invitation.

Mr Olive welcomed Miss Evans back to the council and co-editorship.

Mr Mead outlined the programme for the Conference and he was thanked for his details and for the obvious hard work put into the organisation of the conference.

Minutes of the previous meeting.

Moved:—That they be taken as read.

Business arising out of the minutes.

Meeting of sub-committee from N.Z. I.M.L.T. and N.Z. Society of Pathologists which was chaired by Dr. Taylor of Health Department.

Mr Olive reviewed the recommendations arising from that meeting which had been submitted to the Health Department and went on to outline his report from Mr Donnell on the assignment system and its cost.

The implications were that the cost would have to be met by N.Z. I.M.L.T. but were not insurmountable. Mr Olive expressed the thanks of the members for his hard work in compiling his report.

Review of existing committees.

Moved:—That all committees created by the N.Z.A.B. be dissolved.

Moved:—That all members of those committees hereby be thanked for their services. Messrs Bloore and Cameron.

Applications:

Moved:— That the following applications be approved.

Donnell/Bloore.

For Junior Membership:

John F. Piper, Oamaru; Arlene Campbell, Napier; Janice Warren, Napier; William Waterhouse, Napier; J. P. Van Voorthuizen, Christchurch; C. Campbell, Waipukurau; G. Von Dadelszen, Waipukurau; Marianne Strickett, Green Lane; M. J. Courtney, Auckland; T. Wilson, Auckland; J. Gane, Auckland; G. H. Thorne, Auckland; E. M. Bland, Auckland; Dinah Hall, Auckland; Ann Ellett, Auckland; G. John, Auckland; Christine Macedo, Auckland; B. I. Eathorne, Nelson; R. Tucker, Nelson; A. Deacon, Nelson; Jennifer Harding, Green Lane; K. Macfarlane, Green Lane; W. Haigh, Green Lane; Valmay Wadams, Green Lane; A. McStewart, Path. Dept., Dunedin; L. A. Allingham, Rotorua; R. Wood, Wanganui; Margaret Drew, Wanganui; B. Day, Wanganui; Pauline Cophead, Middlemore; Margaret Hughes, Middlemore; Raiwyn Bluck, Middlemore; Marilyn Wrighton, Princess Mary, Auckland; Francis Grieg, Princess Mary, Auckland; Wayne Meyer, Auckland; Jan Wheelhouse, Princess Mary, Auckland; A. James, Auckland; M. J. Beech, Auckland; Diane Horrocks, Auckland; Adrienne Bone, Auckland; Norma Turley, Green Lane Hospital; H. Stunzner, Green Lane Hospital; Eileen Beagley, Green Lane Hospital; Ann Shaw, Green Lane Hospital; A. Loader, Green Lane Hospital; Miss M. Daly, c/- Dr. Godfrey, Christchurch; Diane R. Sweeney c/- Dr. C. Pearson, Christchurch; Miss J. E. Dalling, c/- Dr. C. Pearson, Christchurch; E. P. S. Norman, c/- Dr. C. Pearson, Christchurch.

Senior Membership:

S. W. Entwistle, c/- Dr. Godfrey, Christchurch.

A. Johnston, Laboratory, Stratford.

Treasurer's Report.

Mr Olive commended the treasurer for having the Balance Sheet regularised.

Moved:—That the treasurers Report and Balance Sheet be received and adopted. Philip/Scarf.

Moved:—That the expenses of the Council meeting be paid. Donnell/Cameron.

ANNUAL REPORT.

Moved:—That the annual Report be approved. Donnell/Bloore.

Correspondence:

The approval of the Council was sought for the formation of the Wellington Branch of the N.Z. I.M.L.T.

Moved:—That the formation of the Wellington Branch of the N.Z. I.M.L.T. be recognised. Donnell/Walker.

Moved:—That the correspondence be received and outward correspondence be approved. Mattingly/Philip.

Common Seal.

Moved:—That the existing common seal still remain but with the appropriate change of wording and that all contributors to the competition be thanked for their efforts. Donnell/Cameron.

The meeting adjourned at 6 p.m. for dinner.

The meeting resumed at 7.30 p.m.

Journal Report.

The co-editor reported that 450 copies were now printed at each issue. Some issues have been thin due to lack of material, the main supply coming from Conference.

Moved:—That the Journal Report be received and that the Editor be thanked for his increasing work with inadequate material. That Branches be asked to encourage further activity in support of the journal.

Remits:—

Remits submitted were perused and put forward for Conference.

Moved:—That further copies of the Branch Rules be cyclostyled and be available as required and that such rules be those of the Auckland Branch as approved by the Council. Bloore/Mattingly.

Salary Submissions.

Moved:—That the salary scales last proposed to the Salaries Advisory Committee with the appropriate adjustments relative to recent alterations from general wage increases be re-submitted. Donnell/Bloore.

Moved:—That consideration be given to the minimum grading of positions available in hospitals and that personal gradings be additional and separate to that of the position. Hutchings/Philip.

Moved:—That the overtime be replaced by a flat on call rate of 15/- per day to be paid to all Medical Technologists on call. Hutchings/Scarf.

The above motion was not carried.

Moved:—That the above motion be brought forward for discussion at Conference. Mattingly/Philip.

The meeting closed with a motion of thanks to the chair at 9.45 p.m.

MINUTES OF THE SEVENTH ANNUAL CONFERENCE OF THE N.Z. I.M.L.T.

Held at New Plymouth Hospital on June 15th and 16th, 1961.

The meeting was opened at 9.35 a.m. by Mr Olive introducing the guests.

Mr Staynton, Chairman of the Taranaki Hospital Board for 28 years, declared it an honour and a privilege to have the Conference at New Plymouth. He hoped that friendship would be achieved and others renewed. Mr Staynton set the tenor of the Conference by warmly welcoming all the delegates present.

In his opening address, Dr. Allen, Pathologist for New Plymouth Hospital Laboratory, pointed out that Laboratory life as an ancillary to medicine has a lot to recommend it. It was, he said, a fascinating and rewarding profession with a worthwhile goal. The responsibility was shared by younger or junior members as by all of the Laboratory staff. Laboratory investigation, Dr. Allen said, was a frontier of science and all concerned could be called scientists despite held opinion that university education used to be required. As a result, the abandoned name was convenient and understood. Our new name was, Dr. Allen felt unsatisfactory, making little difference to the esteem which was dependent on prestige.

Dr. Allen concluded by declaring the conference open.

Mr Olive thanked Dr. Allen for his opening address and explained that the change in name was made to bring our Institute into line with world terminology.

Roll call: B. Main, Dunedin; J. A. Walker, Christchurch; D. G. Till, N.H.I., Wellington; H. G. Bloore, Blenheim; G. Cameron, D. J. Philip, Auckland; Patricia Scarf, Nelson; Joan Mattingly, Miss I. Jarmolicz, Rani Ranger, Margaret Burnett, Linda Wills, Mary Edwards, Wellington; B. Robertson, Thames; A. Johnson, Stratford; Marilyn Eales, Mary Hudson, Lois Evans, Robyn Jenkins, Christchurch; J. Morgan, H. Shott, R. Allen, J. Case, Dunedin; M. Donnell, Auckland; W. Joyce, Waipukurau; J. Callaghan, Auckland; G. Kuru, Wairoa; Ruth Mackie, J. Lyons, Wanganui; Yvonne Young, Napier; J. W. Carroll, Hastings; H. Foster, Lower Hutt; W. G. Orbell, D. J. Weatherby, Auckland; R. Wales, Napier; Miss A. Dunnachie, Wellington; D. C. Smith, Tauranga; I. R. Buxton, Oamaru; G. George, Rotorua; B. Drayton, T. Logan, N. J. Campbell, Waikato; R. McKenzie, Masterton; T. E. Miller, Auckland; S. G. Marshall, Hamilton; W. Poole, Wallaceville; B. Dawkins, D. N. Thorburn, M. McCarthy, Kathryn Schollum, J. Meredith, Auckland; J. E. Horner, Ashburton; W. Aldridge, Balclutha; R. W. Smail, Invercargill; A. L. Schwass, Wellington; Rose Doran, Tauranga; Gail Whitefield, Barbara Pidd, Kaitaia; D. A. Woodhouse, Lorraine Wright, Hawera; A. H. Williams, L. Margolin, H. Hutchings, Palmerston North; K. B. Ronald, Whangarei; J. L. Morrow, Lower Hutt; L. R. Taylor, Wellington; June Gray, C. Thompson, Invercargill; P. J. Bird, V. A. Bâiley, J. M. Taylor, I. W. Saunders, G. D. C. Meads, R. E. Olsen, M. J. Grey, New Plymouth; Str. M. Paula, Str. M. Killian, J. P. Walsh, Auckland.

Apologies were received from: Mr G. W. McKinlay, Waipukurau, Mr L. Reynolds, Wellington; Mr R. McKenzie, Auckland; Mr F. Austin, Dunedin; Mr G. Rose, Christchurch; Mr R. Kennedy, Auckland; Mr G. Tait, Wellington; Mr M. Jenner, Christchurch.

Common Seal:

Moved:—That the recommendation of council that the common seal remain as in the past but with appropriate change in wording be adopted. Shott/Evans.

Remits:

From N.Z.I.M.L.T. Dunedin Branch.

1. That the N.Z.I.M.L.T. should seek authority to conduct its own examinations.

Mr Allen outlined the remit pointing out the need to increase the status and responsibility of the Institute. In view of present progress and the recommendation now in the hands of the Health Department he asked that the remit be withdrawn.

2. That the examination system be revised with the emphasis on specialisation.

Mr Shott congratulated the council on the progress of affairs and asked that this remit also be withdrawn.

3. That simultaneous examinations set by the panel be held in two or more cities.

Mr Shott asked that consideration of this remit be given by the council.

Mr Olive said that the formation of an examining Board as recommended to the Health Department would consider such a problem.

4. That the question of regional representation to council be considered. Mr Case felt that a minority group could be representative of council and suggested dividing country into four regions.

Mr Bloore declared it would be an improvement in some ways but pointed out that working knowledge of Council and Institute affairs requires several years to acquire.

Mr Meredith and Mr Walker felt that Auckland and Christchurch members would be in favour.

Moved:—That the remit be put. Smith/Aldridge.

Moved:—That the remit be considered by Council. Morgan/Shott.

5. That all remits be circulated to members with the notice of the Annual General Meeting.

Moved:—That the remit be put. Morgan/Meredith.

Moved:—That where possible all remits be circulated to members; that remits for circulation to members be in the hands of the Secretary 60 days prior to the Annual General Meeting.

Moved:—That the Conference instruct Council to ensure that proxies may be applied only to remits which have previously been circulated to members prior to the Annual General Meeting. Bloore/Mattingly.

Remits from Wellington.

1. That the Health Department be asked for approval and support for local or overseas Sabbatical Leave for senior members of the Institute, such leave to be for the purposes of further study.

Miss Mattingly pointed out that Scientific officers and Pathologists were granted such leave and such opportunities would be invaluable to our profession.

Moved:—That the Health Department be asked for approval and support for local and overseas Sabbatical leave for senior members of the Institute, such leave to be for the purpose of further study.
Mattingly/Schwass.

2. That the regulation be amended by the deletion of the words Hospital Bacteriologist and its substitution by the words Medical Laboratory Technologists.

Mr Schwass pointed out the need for change in terminology to be in conjunction with the change in name of the Institute.

Moved:—That the Health Department be requested to amend the regulations by the deletion of the words Hospital Bacteriologists and its substitution by the words Medical Laboratory Technologist.

Schwass/Parker.

Auckland remits.

1. Amendment to rule 13 (g) clause 1. That the words two thirds ($\frac{2}{3}$) be deleted.

Mr Meredith felt that the *majority* of the members present whether in person or by proxy should be able to decide the issue of note.

Moved:—That the remit go forward for further consideration.

Meredith/McCartney.

2. That Auckland expresses concern over the lack of information available to members in regard to the Institute's affairs.

Mr Meredith asked for more frequent news letters and that they contain more information.

Mr Olive explained that News Letters could not be more frequent than council meetings and only completed moves and actions could reasonably be published.

Mr Meredith withdrew the remit.

3. Had to be dealt with under remit No. 5 for Dunedin.

Honoraria.

Moved:—That the honoraria remain the same.

Donnell/Mattingly.

Subscriptions.

Moved:—That the subscriptions be £2/2/0 for senior members and £1/1/0 for Junior members. Olive/Philip.

Moved:—That the sympathy of the floor of the Conference be extended Mr Harper. Bloore/Schwass.

Moved:—That the conference committee and the dietetic staff of the New Plymouth Hospital be extended the thanks of the delegates.

Scarf/Walsh.

Mr Donnell extended an invitation from the Medical Services Laboratories for the Conference in 1962.

Mr Meredith extended an invitation from the Auckland Hospital Board.

The invitation of Medical Services Laboratories was gratefully accepted providing the Health Department approved the expenses to such a Conference.

The offer of assistance of Auckland Hospital Staff extended by Mr Meredith to the 1962 hosts in the event of the Conference arrangements being approved was gratefully received.

Moved:—That a vote of thanks be offered the chair.

Donnell/Walker.

The meeting closed at 6.5 p.m.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

Wednesday, 15th March, 1961.

Examiners: Dr. M. McKellar, Dr. F. J. Gwynne, Mr G. W. McKinley.

WRITTEN EXAMINATION

Time allowed three hours.

Answer ALL Questions.

1. The second baby born to a woman, now pregnant for the third time, required an exchange transfusion for erythroblastosis fetalis. Describe fully the investigations you would carry out during the third pregnancy to predict with reasonable confidence that the third foetus is or is not erythroblastotic.
   Discuss briefly how it would be possible to ascertain during the pregnancy that the foetus was definitely erythroblastotic.
2. Describe concisely how the erythrocyte osmotic fragility test is carried out and how the results are expressed graphically. Discuss the rationale of the test and mention briefly the deviations from normal you might expect to observe when carrying out the test on blood from typical cases of —
   (a) hereditary spherocytosis, and
   (b) microcytic hypochromic anaemia.
   What explanations can you offer to account for the deviations from normal in these two disorders?
3. (a) Define the meaning of the term "clearing" in the preparation of histological material for paraffin embedding.
   (b) Describe briefly the morphological characteristics of Cryptococcus neiformans.
   (c) Give an account of the morphology and cultural features of Brucella abortus. By what additional laboratory procedure can infection with this organism be diagnosed?
   (d) Describe briefly your procedure in the performance of a male frog test for pregnancy. Mention any sources of error in the test that you know of.
4. (a) What methods are available for estimating the serum cholesterol level? Describe in detail the method you are accustomed to use. What is your opinion of the accuracy of this method, expressed as a percentage for the normal range?
   (b) Discuss the estimation of serum enzymes, detailing only the principles involved. Describe the details of your method in any one enzyme that you are experienced in estimating.
5. You are given a swab to examine from an infected wound. On clinical grounds the patient is suspected of being a gas gangrene case. Detail the procedure you would follow to give a complete report on the nature of the infection present.
6. Write BRIEF notes on the following:—
   (a) The principles of flame photometry.
   What is the range of normal values for serum Na and serum K.
   (b) Antigen, Antibody, Haptenes, Forssmann antigens.
   (Definition only is required).
   (c) How would you sterilise:
      (i) A container of cotton wool swabs.
      (ii) An intravenous solution of 5% Dextrose 1% Saline.
      (iii) A length of rubber tubing, to ensure internal and external surface sterilisation.

PRACTICAL I

Wednesday, 15th March, 1961

2.30 p.m. - 4.00 p.m. and 4.00 p.m. - 5.30 p.m.

BACTERIOLOGY I:

1. Slide A is a smear prepared from a concentrate of a sputum. The patient is suspected to have tuberculosis. Stain the smear appropriately, and report. Detail staining technique, and write brief notes on a suitable method of concentration.

   (M. tuberculosis)
2. The culture on Plate B, was grown from a child aged 3 with diarrhoea and suppurative arthritis. The organism was present in both faeces and an affected joint. Identify this organism as far as possible. Complete in Practical 3.

   (S. cholerae-suis)
3. C is the catheter specimen of urine from a patient suffering from cystitis. Report on the formed elements in the deposit, and isolate the causative organism. Complete in Practical 3.

   (White Cells. Strep faecalis)
4. The serum D is from a patient with undulant fever. Titre the serum against the appropriate bacterial suspension.

   (High Titre Brucellosis)

BIOCHEMISTRY I:

1. Determine the calcium concentration of the serum X.
   Precipitate overnight, and complete your estimation to-morrow.
2. Determine the Alkaline Phosphatase of the serum Y by the King Armstrong method. Describe the principles involved in the test.
3. (a) Write *brief* notes on the equipment and reagents set out indicating the use of each:
   (a) Van Slyke apparatus.
   (b) McIntosh and Fildes jar.
   (c) Evans blue dye.
   (d) Volumetric flask.
   (e) Selenium barrier layer photocell.

   (b) A specimen of plasma contains 585 mgm. Na C1 per cent. What is this value expressed as milli-equivalents?

   Na = 23 C1 = 35.5

PRACTICAL II

Thursday, 16th March, 1961

9.30 a.m. - 11.00 a.m. and 11.00 a.m. - 12.30 p.m.

HAEMATOLOGY I:

1. Carry out haemoglobin estimation, white cell, differential and platelet counts on the specimen of blood X provided. Detail the platelet method you have used and give the normal range of values.
   Report on the film. (Diagnosis not required).
2. Carry out a "Direct Coomb's Test" on the blood Y. What is the essential serological difference between the direct and indirect Coomb's tests and how should the direct Coomb's test be ideally carried out?

HAEMATOLOGY II:

1. Cross match the recipient's blood C with the donor's blood D provided, in as adequate manner as time permits. Do you feel as a result of your tests that the donor's blood is suitable for transfusion into the recipient?
   Detail your answer.

2. With the A_1 and A_2 bloods provided, determine the titre of anti-A in saline of the serum E.

PRACTICAL III

Thursday, 16th March, 1961

2.30 p.m. - 4.00 p.m. and 4.00 p.m. - 5.30 p.m.

BACTERIOLOGY II:

1. Complete Questions 2, 3 and 4 from 1st Practical.
2. Describe the principles of dark ground illumination microscopy. Use diagrams if necessary, and list the equipment you would require. Why is this method used to demonstrate Treponema Pallidum?
3. Illustrate by simple diagrams, the microscopic appearances of:—
   (a) A hydatid scolex.
   (b) Ascaris lumbricoides.
   (c) Microfilaria in a stained blood film.
   (d) Entamoeba histolytica in a fresh faeces sample.
   (e) Candida albicans.

BIOCHEMISTRY II:

4. Complete Question 1 from yesterday.
5. Test the urine Z for Ketone bodies, sugar, bilirubin and urobilinogen.
6. What does Lambert's Law state?
   What does Beer's Law state.

ORAL EXAMINATIONS:

Dr. McKellar

Significance of Howell Jolly Bodies and Target Cells. Characteristics of well spread blood films. The significance of the / in a genotype (e.g. cde/cde). Crossing over in chromosomes. Chiasmata in chromosomes. Testing for haemoglobin in urine other than spectrosscopically. Trapped plasma. Cold agglutinins. Anti P. Coomb's Test. DU antigen. Saline cross match. Enzyme treated cells.

Dr. Gwynne

Leptospira. Virus culture. Koch's postulates. Skin tests. Echinococcus. Microfilaria. Koch's bacillus. Phage typing. Amboceptor in complement fixation. Theory of complement fixation.

Mr McKinley

Thermometer. Gastric function tests. Liver function tests. Aerobic sporing bacilli. Water testing for bacteria. Milli-equivalents. Anaerobic sporing bacilli. Normal solutions.

Successful Candidates:

Mr J. L. Allen,Auckland.
Miss T. J. Behrens, Wellington.
Miss M. M. Black, Wellington.
Miss M. A. Butcher, Dunedin.
Miss A. E. Ford, Invercargill.
Miss L. A. Mortleman, Wellington.
Mrs F. Narev, Auckland.
Miss H. J. Rawson, Christchurch.
Miss K. M. Schollum, Auckland.
Miss M. B. Scully, Wellington.
Mr C. W. Small, Auckland.
Miss A. L. Turner, Hamilton.
Miss V. E. Wilson, Wellington.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

Tuesday, 21st March, 1961

Examiners: Dr. M. G. Somerville, Dr. F. Smith, Mr L. Reynolds.

WRITTEN EXAMINATION

Time allowed three hours.

Answer ALL Questions.

All questions are of the same value.

1. What bacteriological observations would be of aid in the establishment of a diagnosis of:
   (a) Subacute bacterial endocarditis.
   (b) Pulmonary tuberculosis.
   (c) Bacillary dysentry.
2. What are the reagents used in the Complement Fixation Test?
   What part does each play and what is the source of each reagent?
3. Writes notes on:
   (a) Haemotoxylin.
   (b) Decalcification of tissue.
   (c) Fixation of histological specimens.
4. A patient has received an incompatible blood transfusion —
   (a) What laboratory procedure would you undertake to investigate the mishap?
   (b) What precautions are necessary to prevent such an event happening?
5. Describe the instruments available for measuring the optical density of solutions as applied to biochemical estimations. What are their advantages and disadvantages?
6. Write short notes on any five of the following:
   1. Milli-equivalents.
   2. Formaldehyde stable acid phosphatase.
   3. Somogyi unit.
   4. Folin Ciocalteua reagent.
   5. Calcium oxalate.
   6. Occult blood.
   7. Anthocyanins.
   8. Zinc sulphate turbidity test.

PRACTICAL I

Tuesday, 21st March, 1961

2.30 p.m. - 5.30 p.m.

BACTERIOLOGY:

Candidates will be required to examine 18 spots for which 10 minutes will be allowed for each. During this period candidates are required to observe specimens provided and record briefly the facts required.

SPOT 1: You are provided with six articles for sterilisation:

1. Rubber gloves.
2. Talcum powder; 8 ounces.
3. Talcum powder; ½ ounce.
4. Vaseline; 2 ounces.
5. Record syringe.
6. Clinical thermometer.

State the exact times and methods for complete sterilisation of each.

SPOT 2: Six objects commonly used in bacteriological laboratories are provided. Briefly state the function of each: Objects are:—

1. Acid fuchsine.
2. p-Aminodimethylaniline oxalate.
3. Nigrosine.
4. Optochin discs.
5. Latex 0.81.
6. Cardiolipin.

SPOT 3: Three bottles of solutions labelled A, B and C, are issued as suitable for parenteral use.

Examine and comment as to your agreement with this statement.

Bottle labelled D; what micro-organism is used to produce this product.

A. Suitable.
B. Fragments of glass.
C. Caramelised glucose.
D. Leuconostoc mesenteroides.

SPOT 4:

(a) Identify this object. (Millipore filter).
(b) What is it made of?
(c) Briefly record for what bacteriological purposes you would employ this object.

SPOT 5: Two cultures and two moist films are provided.

Examine moist film and culture A which was obtained from a skin scraping taken from an arm lesion.

Examine moist film and culture B which was obtained from a discharging ear.

Record the probable identity of the cultures provided.

Cultures were

A. Microsporum audouini.
B. Aspergillus niger.

SPOT 6: A guinea pig was inoculated with material suspected of containing tubercle bacilli. The animal was found dead after two weeks. At post-mortem, a suppurative lesion was found. You are provided with the following:

Pure cultures of the organism isolated on MacConkey agar, blood agar and plain agar.

One gram smear, and a smear of the heart blood of a mouse inoculated with material from the guinea-pig (a Leishman stain).

Examine and indicate the probable identify of the organism.

Organism was: (Pasteurella pseudotuberculosis).

SPOT 7: The rack of tubes provided consists of doubling dilutions of the patient's serum. Commencing from the left with equal volumes of the patient's serum and saline, plus two volumes of Brucella abortus agglutinable suspension — *examine,* and

(a) Record the titre.
(b) State whether the titre is diagnostic, and any further points noticed.

SPOT 8: Two slides are provided.

A. Examine and identify, record proper name and any disease with which it is associated.
B. Examine and identify by proper name.

A — Pulex irritans.
B — Pediculus humanus var corporis.

SPOT 9: An anaerobic Gram positive bacillus has been isolated from a leg wound. A pure culture has been used to inoculate the plate provided.

A. What name is given to this type of plate?

B. Describe the reactions taking place.

(A — Nagler plate)

SPOT 10: You are provided with pure cultures of an organism isolated from a patient's sputum.

(a) Blood agar plate after 72 hours incubation at 37°C.

(b) Petragnani slope after 72 hours incubation at 37°C.

(c) Brain heart infusion broth after 72 hours incubation at 37°C.

Two stained films are provided:

D. Gram-stained film.

E. Ziehl-Nielsen stained film.

Examine and indicate probable identity of organism.

Organism was: (Mycobacterium phlei).

SPOT 11: You are provided with three agglutinable suspensions.

(a) Indicate the specific disease for the diagnosis of which each is used.

(b) Record the diagnostic titre in each case.

A — Vi suspension.
B — Streptococcus M.G.
C — Proteus O.X.19.

SPOT 12: List the media provided and mention for what specific purpose each is used. Also any special properties of the medium or its ingredigents.

MEDIA: A to F

A — Liver infusion agar.
B — Desoxycholate citrate agar.
C — Tomato juice agar.
D — Dubos broth base.
E — Littman Oxgallagar.
F — Bacto-Tryptone.

SPOT 13: List the media provided and mention for what specific purpose each is used. Also any special properties of the medium, or its ingredigents.

MEDIA: G to L

G — Krumwiede triple sugar agar.
H — Azide dextrose broth.
I — Mueller hinton medium.
J — Eijkman lactose medium.
K — Egg meat medium.
L — T.C. medium 199.

SPOT 14: Six biological products are provided.

(a) For what specific purpose is each used?

(b) From what is each prepared?

A — Frei antigen.
B — Schick test toxin.
C — Anti plague vaccine.
D — B.C.G. vaccine.
E — Tetanus antitoxin.
F — Tetanus toxoid.

SPOT 15: Three parasites are provided for identification.
Slide A: Identify the parasite, stating class and species.
Briefly record the life cycle.
Parasites B and C:
Examine and identify, stating the Class and species.
A — Echinococcus granulosus.
B — Taenia saginata.
C — Ascaris lumbricoides.

SPOT 16: You are provided with a suspension of killed bacteria (S. typhi). Using the Opacity tubes provided, estimate the number or organisms present per cc.
Calculate the amount of suspension required to prepare 20 ccs. of a vaccine containing 2,000 million organisms per one cc.
(Aseptic technique is not required for this exercise).

SPOT 17: Two organisms A and B have been isolated from cases of gastro-enteritis. Two sets of peptone water sugars with Phenol Red as the indicator are provided.
Sugars from the left:
Lactose, Glucose, Mannite, Saccharose, Salicin.
Organism A is non-motile and Indole negative.
Organism B is motile and Indole negative.
Examine and briefly record any conclusions you are able to draw from this information.

SPOT 18: The moist film on the microscope stage represents the deposit obtained from the centrifuged urine of a patient with P.U.O.
(a) What disease is the patient suffering from?
(b) What further use would the urine deposit be to confirm the diagnosis.
(a) — Leptospirosis.

PRACTICAL II

Wednesday, 22nd March, 1961

9.30 a.m. - 11.00 a.m. and 11.00 a.m. - 12.30 p.m.

HAEMATOLOGY I and HISTOLOGY:

1. Stain and mount the given paraffin section of tissue fixed in formalin by Van Gieson's method.
   Give brief details of method used.
2. Stain blood film A and give brief details of method used. Do a differential white count and comment on the red blood cells.
   Examine and comment on stained films B and C.

A. Hypochromic anaemia.
B. Eosinophilia.
C. Acute leukaemia.

HAEMATOLOGY II:

1. Determine the titre in saline (and give brief account of method used) of the anti-B serum with the B cells provided.
   Is such a serum suitable for ABO grouping?
   How would you preserve such a serum?
2. (a) Do an absolute reticulocyte count using a dry slide technique on specimen (RR).
   (b) Outline one method you would use to perform a coagulation time on blood.

PRACTICAL III
Wednesday, 22nd March, 1961
2.30 p.m. - 4.00 p.m. and 4.00 p.m. - 5.30 p.m.

BIOCHEMISTRY I:

1. Perform a calcium estimation on serum labelled A. Clearly put down in writing the principles of the test, how you performed it and arrived at your result.
2. Perform the usual routine biochemical tests on the urine labelled B. Also examine the deposit.
   Tabulate your results.
3. Six spots are provided labelled 1 - 6; write brief notes on each.
   1. Green filter.
   2. Selenium cell.
   3. 4 - Amino antipyrine.
   4. Folin and Wu blood sugar tube.
   5. S.G. bottle.
   6. Dimethyl aminobenzaldehyde.

BIOCHEMISTRY II:

1. Perform a thymol turbidity test on serum labelled C.
   How is the thymol buffer made up?
   Describe the use of the test clinically.
2. Perform a bilirubin estimation on serum labelled D.
   Put down on paper the principle of the test and your result (detailed workings not required).
   What does the Diazo reagent consist of?
3. Six spots are provided labelled 1 - 6. Write brief notes on each.
   1. Transfer pipettes.
   2. Blowout pipettes.
   3. Sintered glass filter.
   4. Potassium oxalate.
   5. Kjeldahl flask.
   6. Congo red.

ORAL EXAMINATIONS:—

DR. SOMERVILLE.
Anthocyanins, Grey wedge photometer,
Bilirubin, Electrophoresis.

DR. SMITH.
Reticulum stain, Urease, Cold agglutinins,
Sterilization, Liver function tests, Paul Bunnells.

MR REYNOLDS.
Compensating eyepieces, Haemophilus, P.M. Appearances in Laboratory animals, C. reactive protein, T.B. Sensitivities, Dark-ground condenser, Production of penicillinase.

The following Candidates were successful:

Miss M. I. Campbell, Hamilton.
Mr B. F. Dawkins, Auckland.
Miss R. A. Healey, Auckland.
Miss G. R. Walton, Auckland.
Miss M. A. Harman, Wellington.
Miss M. A. Middleweek, Wellington.
Mr J. L. Morrow, Wellington.
Mr W. Hodgson, Gisborne.
Miss P. M. Scarf, Christchurch.
Miss L. A. Scarth, Christchurch.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Examiners: Dr. F. W. Gunz; Mr N. J. Ellison.

Wednesday, 12th April, 1961

WRITTEN EXAMINATION

Time allowed: 3 hours.

Answer ALL Questions.

1. (a) A new microscope has arrived from the manufacturers. Describe how you would align the optical path in order to get the best performance.
   (b) Why are colour filters used in colorimeters and photometers? Give two examples of their use.
   (c) Explain with the aid of a diagram the principle of an electric water still.
2. How do you measure:—
   (a) The coagulation (clotting) time;
   (b) The bleeding time?
   (c) The erythrocyte sedimentation rate.
   Describe one technique for each estimation.
3. A patient has been given a blood transfusion and is reported to have had a "reaction". Write a short essay on the possible causes and prevention.
4. What methods would you use for the laboratory identification of:—
   (a) M. tuberculosis in sputum;
   (b) C. diphtheriae in a throat swab.
5. Describe briefly:—
   (a) The estimation of glucose in cerebro-spinal fluid;
   (b) The estimation of glucose in urine;
   (c) A test for urobilinogen in urine;
   (d) A test for acetone in urine.
6. What laboratory tests would you carry out on a sample of milk to ensure its suitability for human consumption?

PRACTICAL I

Wednesday, 12th April, 1961, 2.30 p.m. - 5.30 p.m.

BACTERIOLOGY

SECTION 1A:

1. Carry out a routine analysis and culture of the urine A provided.
2. Examine and culture the swab B and identify as far as possible the organisms present.
3. Identify the organisms on plate cultures C and D.

Bacteriology to be completed to-morrow.

BIOCHEMISTRY

SECTION 2:

1. Estimate the glucose content of blood X provided.
2. Determine the normality of an approximately N/10 sodium hydroxide solution S. State how you would prepare from this solution a litre of accurate N/10 sodium hydroxide.

PRACTICAL II

Thursday, 13th April, 1961, 9.30 a.m. - 12.30 p.m.

BACTERIOLOGY

SECTION 1B:

Complete bacteriology and report on six "spots".

HAEMATOLOGY

SECTION 3:

1. Specimen A is the blood of a patient in need of transfusion. Specimens B, C, D, E and F belong to potential donors.
   (a) Determine the ABO and D group of specimen A.
   (b) Find which donors are compatible with specimen A.
2. Perform a total and differential leucocyte count on specimen G. Leave the films which you have made. Comment on any features in the films which you think important.
3. Report on films H, I, J, K, L, M.

ORALS:

Microscopes, P.C.V., Coombs Test, Transfusion reactions, T.N.P.N.'s, Anaphylaxis, Hypobromite reaction, ureometers, Media, Anaerobiosis, Fouchet's reagent, Normal solutions, Coagulase, Esbach reagent, Sterilisation..

The following Candidates were successful:

Miss V. D. Bailey, New Plymouth.

Mr R. Coleman, Wanganui.

Mr L. Cross, Gisborne.

Miss D. E. Withy, Gisborne.

Miss A. M. Devonshire, Hamilton.

Mr E. Kingsley Fletcher, Medical School, Dunedin.

Miss A. M. Foley, Christchurch.

Mr M. J. Grattan, Christchurch.

Miss V. M. Tucker, Christchurch.

Miss U. E. Montgomerie, Palmerston North.

Mr A. H. Williams, Palmerston North.

Miss J. Bisset Smith, Masterton.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Examiners: Dr. A. F. Burry, Mr A. L. Schwass.
Wednesday, 26th April, 1961
9.30 a.m. - 12.30 p.m.

WRITTEN EXAMINATION
Time allowed 3 hours.
(All Questions Carry Equal Marks)

Question 1:

You are presented with a bottle containing 200 ml. of slightly turbid, Describe in detail your procedure in examining this fluid and the order in which you would carry it out.
Name the main ingredients of any reagents, stains or media you would use and mention their purpose.

Question 2:

Give very brief answers to the following:
(a) From what cell are platelets formed?
(b) Name four types of casts found in urine.
(c) Draw a typical ammonium urate crystal.
(d) Give the chemical equation in the hypobromite reaction.
(e) What is the action of E.D.T.A.?
(f) What is the depth of a Fuchs' Rosenthal counting chamber?
(g) Name two characteristics of a reticulocyte as *stained by Leishman* only.
(h) What proportions of blood and citrate are used to fill a Westergren tube?
(i) Draw and name the tube used to carry out a coagulation time in a baby.
(j) What are the reagents used for Folin-Wu precipitation of blood proteins?
(k) How is starch detected in a fractional test meal?
(l) Which is the more sensitive: Gregerson's test or the Benzedine test?
(m) Name two methods of detecting protein in urine.
(n) Name the constituents of a quantitative reagent for estimating urine albumin.
(o) Give the normal range for absolute lymphocyte count in an adult.
(p) What pigment is measured in haemoglobinometry, using the M.R.C. Grey-Wedge Photometer?
(q) Name two differences between eosinophils and neutrophils.
(r) What is the reason for the shape of a Folin tube?
(s) How would you treat a case of electrocution?
(t) How would you treat a caustic soda burn of the mouth?
(u) What is the area of the smallest square in a Neubauer chamber?
(v) What colour is Topfer's reagent at pH 4.5?
(w) Name three factors which influence the time a specimen of blood must be centrifuged to establish the P.C.V.
(x) Name three cells which may be found in C.S.F.

Question 3:

Describe the methods of sterilization commonly used in the laboratory. Give the principle, and an example of the use, of each method.

Question 4:

Answer briefly any *FOUR* of the following:

(a) What are Pyrogens? How would you avoid their presence in parenteral solutions?

(b) Draw a simple diagram to illustrate the workings of a photoelectric colorimeter. State Beer's Law.

(c) For what purpose are the following media used? List their ingredients:

(1) Bordet Gengou.
(2) Tetrathionate broth.
(3) Hoyle's tellurite.

(d) A streptococcus isolated from a specimen of sputum showed alpha haemolysis. List the methods you would employ to identify the species. (Details of the methods are not required).

(e) An organism of the enteric group has been isolated. List the tests (serological tests are NOT required) you would employ to identify the organism. (Details of the methods are NOT required).

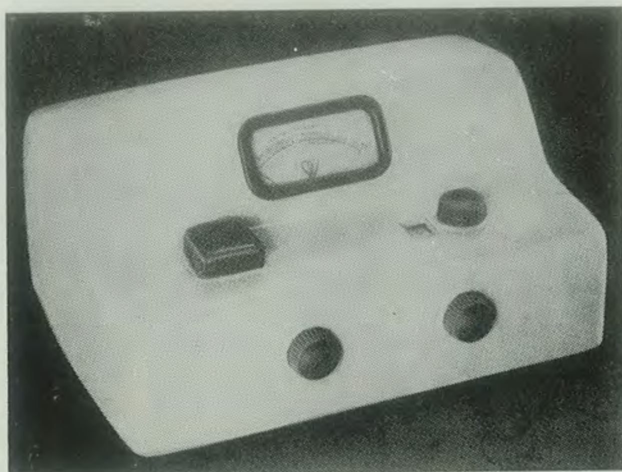
ORAL EXAMINATION:

Incomplete antibodies, Coombs Test, Cross matching procedures, Colorimeters, Cross matching procedures, Absolute values, Coloured filters.

NO PRACTICAL EXAMINATION WAS REQUIRED.

SUCCESSFUL CANDIDATES:

Miss E. A. Beagley, Auckland.
Mr M. S. G. Clist, Auckland.
Mr D. A. Fisher, Auckland.
Mr D. G. Henwood, Auckland.
Mr T. B. Martin, Auckland.
Mr M. R. Peters, Auckland.
Mr O. R. Phillips, Auckland.
Mr G. D. De Silva, Auckland.
Mr R. Spiers, Auckland.
Mr W. J. Wiggle, Auckland.
Mr D. A. McArthur, Auckland.
Miss J. E. Gresham, Wellington.
Miss J. A. O'Grady, Wellington.
Mr I. H. Symonds, Wellington.



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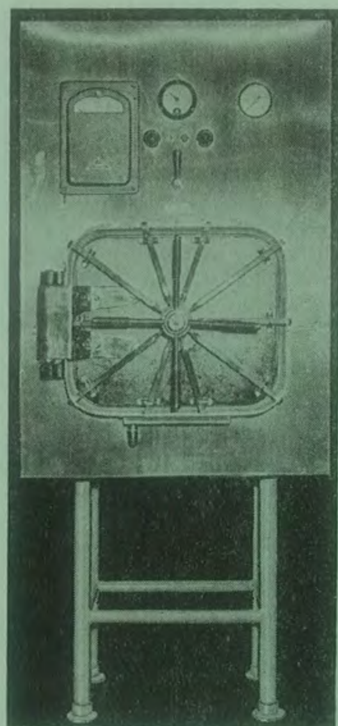

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