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Modifications to the SMA 12/60 to Enhance Productivity

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Received for publication, October 1976

Summary

This paper describes simple modifications to an SMA* 12/60 that reduce sample requirements from 1.8ml to 0.8ml. This was accomplished by modifying the urea dialyser and resampling both streams. It resulted in a significant cost benefit due to a reduction in the use of reference serum and re-collection of samples.

Introduction

The SMA 12/60 is widely accepted in the clinical laboratory. Perhaps the most significant benefit of this sequential multiple analyser is work flow simplification; approximately 60 percent of Chemical Pathology patient test requests require minimal specimen preparation, are processed at one work-station and patient collated reports are produced, thereby eliminating much manual effort.

A review of the first year of operation showed the most significant costs to be staff salaries, quality control sera, reagents and manifold pump tubes, in that order.

The overall objective of improving the productivity of the SMA 12/60 was to be based on the development of a three phase strategy.

1 Reduce sample size, reagent costs and manifold tubes.

2 Increase test parameters from 12 to 15 utilising separate recorders to save technical time, and still retain reduced sample size and minimal sample preparation.

3 Using the process control capabilities of the Nationwide Clinical Laboratory Computer System to take full advantage of the enhanced SMA to increase the analytical rate.

The following is a report on phase 1.

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Material and Methods

a. *Reagents for predilution manifold*

1. Donor stream. 0.15M sodium chloride, containing 1 ml 30% Brij 35 per litre.
2. Recipient stream. 0.15M sodium chloride, containing 0.5ml Aerosol-22 per litre.

b. *Quality Control Material*

1. Technicon SMA reference serum 2 SGPT product number T 1308250.
2. Calbiochem cal-trol reference serum catalogue number 30002.
3. Pooled bovine serum acquired from local abattoir.

c. *Equipment*

SMA 12/60 with analytical cartridge sequence sampler; pump 1 (aspartate aminotransferase, alkaline phosphatase, uric acid); pump 2 (sodium, potassium, carbon dioxide, urea, calcium); pump 3 (total bilirubin, hydroxybutyrate dehydrogenase); pump 4 (albumin, total protein). The new predilution stage utilised the 12-inch dialyser on the urea cartridge. The connector assembly, part number 157-B037, for this cartridge was modified by adding to the right-hand side reagent inlet line both air and resample inlets. This enabled the urea channel to be operated through the modified urea analytical cartridge. Outlets for the predilution dialyser were constructed from 1mm internal diameter glass part number 157-E007-01 and channelled down and behind the connector assembly, along the manifold tray between pumps 2 and 3 and to the appropriate location for sampling.

d. *Method*

(This is illustrated by the flow diagram in figure 1).

Sample is added to 0.15M saline containing Brij 35, mixed in an 8.25 turn mixing coil and passed through a 12-inch dialyser against a recipient stream of 0.15M saline containing Aerosol-22. The appropriate amount from these streams is then resampled.

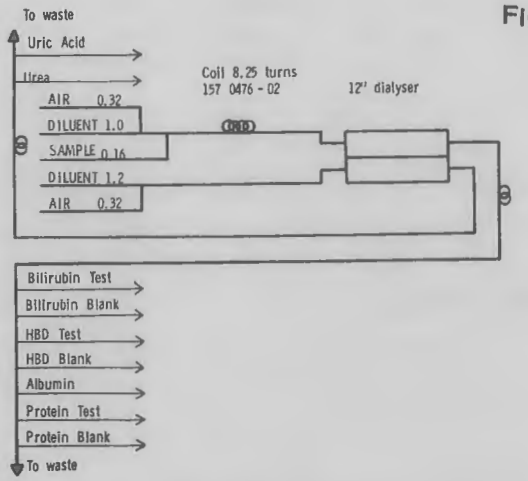

Figure 1. — Flow diagram of new predilution technique.

Figure 2. — Photograph showing modified urea analytical cartridge and connector assembly with additional inlets for air and resample on right-hand side.

TABLE 1

	Sample	Urea mmol/l	T.protein g/l	Albumin g/l	Uric acid mmol/l	T.bilirubin umol/l	H B D U/l
Number of Assays	A	19	22	22	19	22	20
		21	15	17	17	20	17
Mean		1.8	59	37	0.36	49.2	232
		11.2	35	29	0.051	10.1	171
Coefficient of Variation		3.9	2.8	2.4	3.4	2.8	2.6
	n	2.0	1.7	1.8	2.6	3.5	1.9
Standard Deviation		0.07	1.7	0.87	0.012	1.38	6.1
	n	0.22	0.59	0.53	0.013	0.36	3.2
Percent Interaction		1.9	2.9	1.5	1.7	3.1	2.1

Table 1. — Standard deviation, coefficient of variation and percent interaction studies.

Results

The linearity of the 6 chemistries were checked by plotting concentration versus dilution of suitable sera. All demonstrated linearity over the dynamic range for the instrument.

Table 1 summarises the standard deviation, coefficient of variation and percent interaction studies performed on samples A and B. These samples were calf serum and calbiochem reference serum diluted to appropriate concentrations. The results were collected over a period of one week. The apparent discrepancy in the trends of coefficient of variation reflect minor changes in phasing.

Discussion

The results shown in Table 1 demonstrate acceptable performance under normal operating conditions. The modification has been in use for over a year and has not produced any new operating problems.

The rate of dialysis may be improved by preheating the recipient stream, however, sensitivity was adequate without the use of this technique. Dialysis membranes are sensitive to pressure differences across the membrane caused in this case by the nature of the resampling. Gross imbalance may cause partial occlusion of one exit groove resulting in a poor bubble pattern. Glass tubing from the dialyser reduces this effect to tolerable levels. Equal concentrations of Brij 35 on both sides of the membranes is ideal but Aerosol-22 is necessary in the recipient stream to prevent precipitation in the uric acid channel.

While sequential dialysis is an alternative, it was not used because the wash characteristics of multiple 6 and 12-inch dialysers is relatively poor. Further, without major alterations to

the analytical sequence, this method would have created phasing problems, and the length of interlinks between the dialysers would also reduce the benefit of analytical cartridge independence.

The benefits from the phase 1 strategy include:

1. Sample requirement reduced from 1.8ml to to 0.8ml.
2. Quality control sera cost is reduced in proportion to the sample size reduction.
3. One 10ml sample collection tube is sufficient for the majority of patient requests.
4. Repeats for whatever reason are less likely to require re-collection of specimens.
5. Paediatric enzyme determination is possible, thereby overcoming problems associated with multi-instrument variations in method, and result interpretation.

A significant trend in clinical chemistry is minimisation of resource use. In the situation described the cost of quality control sera and the reduction in sample volume were seen as areas suitable for investigation. In this situation the cost benefits were considerable.

Acknowledgments

The author wishes to thank all technical staff for their tolerance during the change-over period.

Evaluation of the Pye Unicam SP30 Reaction Rate System

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Received for publication, December 1976

Introduction

The Pye Unicam SP30 spectrophotometer is sold in New Zealand by Philips Electrical Industries as a medium-priced spectrophotometer in a number of integrated packages. Features include, automatic gain control, photomultiplier detectors and solid state electronics which are claimed to give the instrument stability for long unattended runs, high linearity and low noise. In this evaluation the instrument is evaluated for value for money, comparison of manufacturer's specifications, layout and ease of use and finally a comparison of some methods from other systems.

Instrument Packages

The Pye Unicam SP30 spectrophotometer has a number of integrated packages:

1. SP30 UV, which has a wavelength range of 220 to 750nm and uses a dual deuterium and tungsten lamps.
2. SP30 Visible, which has a wavelength range of 300-750nm and uses a quartz halogen lamp.
3. SP30 Auto Cell System, which uses an automatic sipper device. This can be used on any of the SP30 models and, it is claimed by the manufacturers, will permit the reading of assays up to 300 per hour. The sample volume can be varied.
4. SP30 Reaction Rate System, which comprises a basic SP30 UV or SP30 Visible spectrophotometer to which a reaction rate accessory has been added. The reaction rate accessory will allow the enzyme reaction to be followed with either increasing or decreasing optical density and to obtain readings in either 30 or 60 seconds. It incorporates an automatic zero system which zeros prior to the start of a new series of readings. Results are printed on an accessory printer.

Reaction Rate System

The physical specifications were as follows:

Dimensions	520 × 410 × 330mm
Weight	33kg
Slits	0.35mm etched
Monochromator	Monk-Gillieson, sealed F/4 grating monochromator with focus compensation.
Optics	Double beam with friction-less beam splitter/modulator.
Cuvette size	Variable.

The basic SP30 Visible spectrophotometer with reaction rate accessory and printer is shown in Figure 1. Working from left to right the controls are as follows:

1. Wavelength Control.

The wavelength settings are digital and altered by turning the wavelength control knob.

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- Alkaline phosphatase levels stable immediately after reconstitution.
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The wavelength is fixed by pressing the knob in to lock the display and pulling towards the operator to unlock and change the wavelength display. This was a particularly good feature. The display, however, is variable between 0 and 999 and it was felt that an operator could use the instrument unwittingly outside of the specified wavelength range. This could be overcome by installing wavelength stops.

2. Mode Switch.
   This switch has five functions:
   a. On/Off.
   b. Absorbance reading 0-3 optical Density.
   c. Factor ÷ 10, which enables the appropriate factor to be set to give concentration readouts.
   d. Concentration Rate, which enables direct readings to be made up to four digits with selection of the decimal point place.
   e. °C, which is a temperature readout mode giving a visual display of the current temperature at which the cuvette block is operating. It has a range of 0-100°C and the temperature reading may be recorded on the printer.
3. Digital display unit.
4. Set Concentration/Factor.

Using this control the factor for the assay may be set when the instruments mode switch is set on the Factor ÷ 10 position.

5. Reaction Rate Accessory.

This unit was simple to use and it was felt that few errors could be made operating this unit. The zero button is depressed and the instrument is zeroed using the Zero Set Point. The button is then released and the instrument will then automatically zero itself at the start of each kinetic assay. For reaction rate work the Rate Mode Switch is depressed. Depending on the nature of the reaction, increase or decrease in optical density and 30 or 60 second monitoring time can be selected using these two buttons.

6. The printer is standard DR16 Philips printer which uses a typewriter ribbon for printing. It has an on/off button, a paper advance button and a read button. During operation it was extremely quiet.

Costs and Accessories

The approximate cost breakdown of the instrument and two of the accessories is shown in Table I; it is emphasised, however, that an actual quotation must be obtained from the agents before consideration to purchase.

TABLE 1

		$
SP30 Visible		3,942
Reaction rate accessory		789
Constant temperature holder		177
Temperature readout accessory		177
DR16 digital printer		2,136
	Total	7,221
Tecam C100 circulator		395
	Total	7,616
Autocell accessory		789
	Total	8,405

Table I. — Cost of SP30 Reaction Rate System including the Tecam C100 circulator and the Autocell accessory. All prices are New Zealand dollars.

The three major accessories cited by the manufacturer are a Tecam C100 temperature water circulator, a sampler capable of sampling 50 samples per hour and a recorder. During this evaluation only the Tecam C100 was used. It must be borne in mind that cuvette temperature control facilities in this instrument are sold as an optional extra, therefore, if no temperature water circulation facilities exist in the purchaser's laboratory, this would be extra.

Accessibility and Servicing

On the left-hand side of the sample compartment there is the photomultiplier housing. Access is readily available by removing two thumb screws situated at the bottom of the housing, the cover then slides off and is equally easy to replace. The photomultiplier was found to be easy to replace. The sample compartment is the large black section on the front of the instrument (Figure 1). This houses a thermostated cuvette holder which accepts variable length light path cuvettes and holds a reference and sample cuvette. The temperature monitor is mounted centrally in the cuvette block. Lifting the sample compartment lid operates a microswitch which acts as the photomultiplier on/off switch. Additional access to the sample compartment is gained by removing the front panel of the compartment. This is a good feature as it gives ease of access if coping with spillages.

On the right-hand side of the sample compartment is the lamp and monochromator housing. Access to it is the same as the

Figure I. — SP30 Reaction Rate System with DR16 printer.

photomultiplier housing. The lamp was an air-cooled quartz-halogen; access and replacement was found to be easy. The monochromator is housed in a separate unit to which access can be gained, if required.

Access to the electronic and mechanical components of this instrument were similarly easy. The screws at the back of the instrument were removed and the cover slid off. This again was easy to replace. All the printed circuit boards were of the plug-in type and all components were within easy access for replacement or servicing. There are no controls mounted at the back of the instrument; this is a particularly good feature. All fuses and the voltage regulator were mounted externally, in a recess on the right-hand side of the instrument.

The handbooks for the SP30 Reaction Rate System and the printer were well prepared, although it was felt that they must be read a number of times to completely understand the system.

During the period of evaluation, the instrument required no servicing from the agents. However, Philips do carry a wide range of spares for this model and personal experience with Pye-Unicam equipment servicing has been excellent.

Instrument Performance

The instrument's performance was assessed on the basis of the working party on Laboratory Equipment and Methods Advising Group (1969)[3], the criterion of Georges (1973)[2] and the preliminary recommendations of the working party report "Buying for the National Health Service" (1975)[1].

The performance parameters studied were as follows:

1. Wavelength accuracy.
2. Wavelength reproducibility.
3. Photometric accuracy.
4. Photometric reproducibility.
5. Stray light.

All results are shown in Table II.

1. *Wavelength Accuracy*

This was checked using the spectra of both holmium and didymium filters.

2. *Wavelength Reproducibility*

Wavelength reproducibility was checked by reading the 361nm peak of the holmium filter, thrice, four times a day for five days. Although the wavelength reproducibility was not stated by Pye-Unicam, the observed value of less than 1nm was very satisfactory.

3. *Photometric Accuracy*

This was measured using a 60mg/litre potassium dichromate dissolved in 0.01N sulphuric acid. The photometric accuracy was measured at four different wavelengths.

4. *Photometric Reproducibility*

The photometric reproducibility for the SP30 was not stated; however, the general criterion is usually ± 1%. The procedure as for assessing photometric accuracy was followed, only in this case seven different wavelengths were used. The test solutions were read thrice at each wavelength, each day for five days.

5. *Stray Light*

Stray light was assessed at 325nm using acetone, at 453nm using a holmium filter and at 586nm using a deuterium filter. All three selected wavelengths had optical densities in excess of one Optical Density.

Method Evaluation

The following methods were used to evaluate the instrument's analytical performance: Uric acid; Urea; Alkaline phosphatase; Aspartate aminotransferase; Creatine kinase; α Hyroxybutyrate deyhdrogenase.

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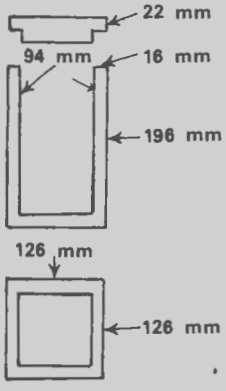

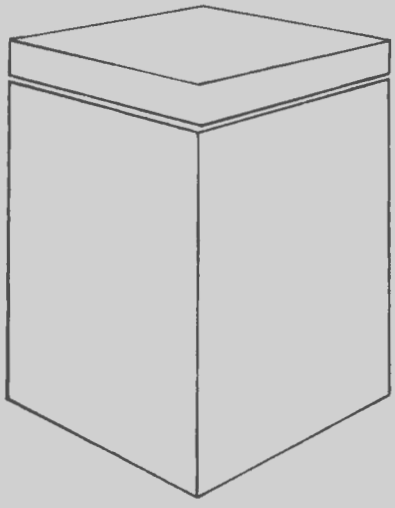

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● PHOTOMETRIC ACCURACY	±1% of ABSORBANCE READING		
340nm		2%	
480nm		0	
510nm		0	
580nm		0.5%	$\bar{x}$=0.63%
● PHOTOMETRIC REPRODUCIBILITY	NOT STATED USUALLY ±1%		
340nm		1.4%	
360nm		0.5%	
400nm		1.2%	
460nm		0.3%	
480nm		0.3%	
510nm		0	
540nm		0.3%	$\bar{x}$=0.57%
● STRAY LIGHT			
325nm	< 0.5%	< 0.5%	
453nm	NOT STATED	1.0%	
586nm	NOT STATED	1.0%	

Table II. — Comparison of manufacturer's SP30 specifications and the observed specifications during evaluation.

All methods for the SP30 evaluation used Calbiochem kitsets. The uric acid, urea, alkaline phosphatase and aspartate aminotransferase methods were all Technicon Auto Analyser methodologies. The creatine kinase and hydroxybutyrate dehydrogenase assays were performed on the Vitatron AKES. The quality control sera used were Dade and Wellcome.

All methods had 20 individual patient tests run simultaneously, and as blinds by a single operator (ML).

With the exception of uric acid, no test had a delay between methods of greater than 24 hours; the uric acids had a delay of 48 hours. To compare the two sets of results the regression line was used.

Assessment of Modes

To assess the use of the 30 second, 60 second and absorption modes, the alkaline phosphatase method was used.

First the full reaction was performed taking 10 minutes for completion of reaction, the absorbances read and the results calculated. The same samples were then analysed in the 60 seconds and 30 second reaction rate modes.

Parameters of the regression lines for reaction rate at 60 seconds against absorbance, 60 seconds against 30 seconds reaction rates and 60 seconds reaction rate against Auto Analyzer were:

$a = 0.41$, $b = 0.959$, $r = 0.9983$; $a = 0.68$, $b = 0.959$, $r = 0.9987$; and $a = 2.36$, $b = 0.208$, $r = 0.921$ respectively.

For all further reaction rate assays, the 60 second reaction rate mode was used.

The parameters of the regression lines are tabulated.

Substance	a	b	r
Uric acid	0.831	0.704	0.9077
HBD	1.63	1.018	0.9989
Urea	0.838	1.026	0.9973
Asp. aminotransferase	0.458	0.474	0.9931
Creatine kinase	0.969	1.017	0.9988

Conclusion

The SP30 Reaction Rate System is a medium-priced versatile reaction rate system which is well suited for the small to medium-sized laboratory. It is a very compact, well-manufactured double beam spectrophotometer. In my laboratory it performed well within the manufacturer's specifications, and test values were very close to the expected values as given by other methods used on alternative systems. The latter point, however, is an arguable criterion when assessing instrumentation whose specifications do not include reagent dispensers systems and/or sample preparation units: and, therefore, need not be a measure of the instrument's capabilities.

To assess the instrument in terms of good and bad features: the good features were found to be, ease of servicing and accessibility to all components, the wavelength lock device, temperature readout, double beam facilities, autozero, the non-dedication of the instrument to a specific function, e.g., reaction rate only, and the overall ease of use. The bad features were: no wavelength stops, the overall change in optical density cannot be monitored when using the reaction rate mode, and a separate temperature control system.

This instrument, however, performed exceptionally well during evaluation and is well suited in a laboratory with a small, varied workload, possibly using the autocell accessory.

Acknowledgments

I am extremely grateful to Philips Electrical Industries (NZ) Ltd for the loan of the SP30 Reaction Rate System during the evaluation period, Calbiochem (NZ) Ltd for supplying reagents and Department of Clinical Biochemistry, Christchurch Hospital, for providing patient samples and results.

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A Beta-Lactamase Producing *Haemophilus influenzae* Type a

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Received for publication, January 1977

Summary

An ampicillin resistant strain of *H. influenzae* was isolated from a young child who had been treated over many years with cloxacillin. Methods to test for the production of beta-lactamase from *H. influenzae* are described.

Introduction

Ampicillin is generally an effective drug for the treatment of haemophilus infections, but there are increasing numbers of reports on ampicillin resistant strains of *H. influenzae*[3, 5, 11, 13, 14].

Resistance is associated with the production of beta-lactamase[3, 9, 10], enzymes that can destroy the antibacterial activity of beta-lactam antibiotics (the Penicillins and Cephalosporins).

The disc diffusion sensitivity test however, may fail to demonstrate resistance to penicillin and ampicillin.

This paper reports on the isolation of an ampicillin resistant strain of *H. influenzae* type a and describes the tests used to detect beta-lactamase production.

Case Notes

A 5-year-old boy with cystic fibrosis was receiving continuous cloxacillin therapy for the control of respiratory infections. His treatment was changed to amoxycillin when otitis media developed. Although the ear cleared on this, there was increasing chest infection with purulent sputum. Cultures of the sputum gave heavy growths of *H. influenzae* and *Staphylococcus aureus*. The *H. influenzae* was sensitive to tetracycline, cotrimoxazole and chloramphenicol, but resistant to penicillin, ampicillin, cephaloridine, erythromycin, lincomycin and cloxacillin by disc diffusion sensitivity tests. An MIC to ampicillin determined by the NHI Reference Laboratory was 8μg/ml. The *Staphylococcus aureus* was sensitive to erythromycin, cotrimoxazole, cephaloridine, cloxacillin, lincomycin and chloramphenicol. Both organisms were shown to produce beta-lactamase. Amoxycillin was discontinued and "Septrin" (cotrimoxazole) was started. This was followed by a gradual improvement in the boy's condition.

Methods

Columbia blood and chocolate agars were used for sputum culture. Morphology of the haemophilus by Gram stain, growth factor requirements for X and V factors and capsular typing were all by routine methods. The Stokes plate technique[2, 8] was employed for the haemophilus sensitivity testing, chocolate agar was used with the sensitive *Staphylococcus aureus* Oxford (NCTC 6571) for a control organism. A light inoculum of *H. influenzae* was used to spread the central portion, standard discs; penicillin 5 unit (3μg), tetracycline 30μg, ampicillin 25μg, erythromycin 15μg, "Septrin" (cotrimoxazole) 25μg, cloxacillin 5μg, cephaloridine 15μg, lincomycin 10μg and chloramphenicol 30μg were used in the tests. All plates were incubated at 37°C overnight in a candle jar.

Demonstration of Beta-Lactamase Production

1. A section of the sensitivity plate was set up to detect the breakdown of penicillin. Light inocula of the control Oxford staphylococcus and *H. influenzae* were spread over adjacent halves of a chocolate agar plate. A 10 unit (6μg) penicillin disc was placed

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Figure 1. — Plate inoculated with beta-lactamase producing *H. influenzae.* The control Oxford staphylococcus has regrown where penicillin has been destroyed.

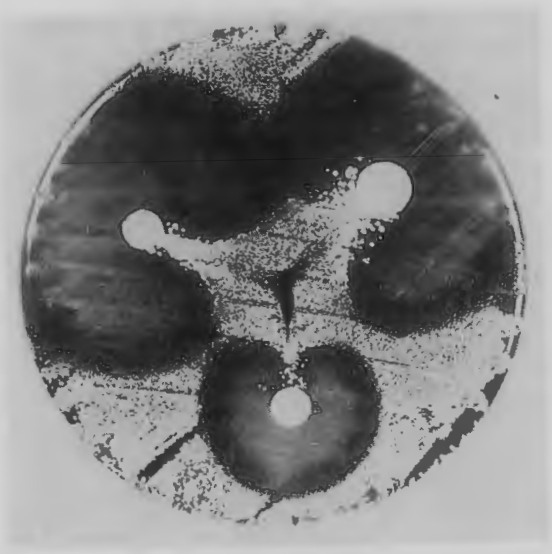

Figure 3. — Plate streaked with beta-lactamase producing *H. influenzae,* showing regrowth of the Oxford staphylococcus along the streak lines.

Figure 2. — Ampicillin sensitive strain as a negative control.

Figure 4. — Ampicillin sensitive strain as a negative control. Discs contain (AP25) ampicillin 25μg (CX 5), cloxacillin 5μg and (blank) penicillin 10 units (6μg).

on to the agar at the junction of the two organisms (Figs. 1 and 2).

2. Destruction of penicillin, ampicillin and cloxacillin was tested for by a modification of the method described by Lee and Komarmy 1976[4]. A chocolate agar plate was seeded with a broth suspension of the control Oxford staphylococcus and the surface dried. Approriate antibiotic discs were placed on to the agar surface, several colonies of *H. influenzae* were then picked off the primary culture and spread radially to the edge of each disc (Figs. 3 and 4). Culture plates for these two tests were also incubated at 37°C in a candle jar.

3. A rapid capillary method[7] was used to detect beta-lactamase production. A penicillin test solution was prepared by adding two drops of 0.5% phenol red solution to 1ml of reconstituted penicillin G (1 million units/ml). 1.0 Molar sodium hydroxide was added drop by drop with a fine Pasteur pipette until the solution turned violet. Capillary tubes were half-filled with this penicillin-phenol red test solution. The tip of the tube was lightly scraped across several haemophilus colonies so that a plug of organism was in contact with the test solution. Tubes were stood vertically for 5-15 minutes and observed for a colour change. Ampicillin sensitive strains of *H. influenzae* were used for a negative control.

Results

The *H. influenzae* gave a 4mm zone of inhibition to the ampicillin 25μg disc, with large colonies forming a heaped up zone edge similar to those obtained from penicillinase producing staphylococci. No zone was obtained with the penicillin 3μg disc. The zone of the control Oxford staphylococcus was altered as illustrated more clearly in Fig. 1. Regrowth of the control organism has occurred where beta-lactamase from the *H. influenzae* has destroyed penicillin.

Fig. 3 shows destruction of penicillin, ampicillin and to a lesser extent cloxacillin, with regrowth of the control organism along the streak lines.

This organism was presumptively called a beta-lactamase producing strain of *H. influenzae* and resistant to ampicillin. Confirmation of beta-lactamase production was obtained by a positive result with the capillary method. The violet colour of the penicillin-phenol red solution changed rapidly to yellow as penicillin was hydrolysed to penicilloic acid. No colour change within 15-30 minutes occurred with ampicillin sensitive strains of *H. influenzae* used as negative controls.

Discussion

The H. influenzae type a isolated from this case was markedly resistant to ampicillin by disc diffusion sensitivity tests. However, there are reports in the literature[6, 12] that the disc diffusion test may be unreliable for testing the antibiotic sensitivity of haemophilus organisms. By also using methods to detect beta-lactamase production, suspected ampicillin resistance can be confirmed.

Resistance of *H. influenzae* type b has only occasionally been reported since 1970. Australian surveys by Bell and Smith 1975[1], give evidence that these strains are present in the community. As yet no amipicillin resistant strains of *H. influenzae* have been reported in New Zealand.

Laboratory staff should be aware that resistant strains may occur and adopt methods for their detection.

Acknowledgments

My thanks to Dr J. F. Burton, also staff at the NHI Reference Laboratory, Wellington, for assistance in preparing this paper. Thanks to Mr J. Poon for the photographs.

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Technique for In Vitro Culture of Haemopoietic Cells

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Based on a paper presented at NZIMLT Conference, Whangarei, 1976.

Received for publication, December 1976

Summary

The technique for the cultivation of haemopoietic cells in a semi-solid medium is used for the quantitation of the granulocyte progenitor cells and at least some of the macrophage progenitors in any haemopoietic tissue. The available data indicates that the colony forming cell is considered to be a committed granulocyte precursor rather than the multipotential stem cell[10]. These haemopoietic precursor cells can be stimulated to divide and form colonies, but in order for these cells to survive and proliferate in vitro, a factor known as the colony stimulating factor ("CSF") must be incorporated into the medium[8]. This culture system has been used to study human disease situations in which granulocyte production is altered or defective, including the leukaemias, neutropenias, and aplastic anaemia[10].

Introduction

The technique for the cultivation of haemopoietic cells in a semi-solid medium was introduced independently, by Pluznik and Sachs 1965[9] and Bradley and Metcalf, 1966[1], using haemopoietic cells from mice. In 1970 Pike and Robinson[6] introduced a modified system for the culture of haemopoietic tissue from humans. This system uses a modified McCoy's 5A medium with 15% foetal calf serum added. The colony stimulating factor is provided by an underlay of human peripheral leucocytes, which is required for the growth of colonies[4].

The number of colonies developing after incubation is dependent upon the number of colony-forming cells plated and the amount of "CSF" present in the underlays[5].

In 1974 Moore *et al*[10] re-classified acute leukaemias (mainly myeloid) according to growth patterns in agar and attached to these growth patterns a prognostic significance in relations to treatment with chemotherapy. This technique was set up in our laboratory so that these patterns of growth of granulopoietic progenitor cells from patients with acute leukaemia might be studied, with the aim of providing additional information to the clinician.

Materials and Method

Preparation of Feeder Layer (Source of "CSF")

Approximately 70ml blood is collected from a normal volunteer with a sterile, disposable butter-fly set into a sterile, capped bottle containing 35ml 6% dextran in saline and 1% heparin solution (sigma-sodium salt, preservative-free) giving a final concentration of 25 units/ml. The blood is well mixed and left to stand at room temperature for approximately half to one hour. The leucocyte content of the plasma is determined using a new improved Neubauer counting chamber. The medium used to prepare the feeder layers and overlays is shown in Table 1. The basis of this medium is a modified preparation of

TABLE I

COMPOSITION OF MEDIUM USED FOR HUMAN BONE MARROW COLONY GROWTH

McCoys 5A medium single strength	800ml
Foetal calf serum	150ml
Sodium Bicarbonate (7.5% soln)	6ml
Sodium Pyruvate (100mM soln)	10ml
Mem Vitamins (100x)	4ml
Mem Amino Acids (50x)	8ml
Mem Non-essential Amino Acids (100x)	4ml
Mem Glutamine (200mM)	4ml
* L — Serine (21mg/ml)	0.4ml
* L - - Asparagine (10mg/ml)	1.6ml

McCoy's 5A medium (Grand Island Biological Company — California) to which is added 15% foetal calf serum (Lab. Serv.—Auckland) and a variety of other elements in optimum concentrations. The McCoy's 5A medium is mixed in a 9:1 concentration with autoclaved 5% agar (Difco-Bacto-Agar) to give a final concentration of 0.5%. The medium must

* Sigma Chemical Company

first be warmed to 37°C prior to the addition of the agar to prevent gelling. The agar is added to the medium at a temperature just below the boiling point. The mixture is left to cool to approximately 40°C and the human leucocytes are added to this mixture to give a final concentration of 1 x 10^6 cells/ml. One millilitre aliquots of this medium-agar-leucocyte mixture are pipetted to 35mm plastic petri dishes (Falcon Plastic, California). These plates are then left to gel at room temperature.

Preparation of Bone Marrow Overlays

The human bone marrow for culture is obtained from sternum or posterior iliac crest. The marrow aspirate for culture is collected by aspirating 2-4ml of bone marrow into a sterile capped bottle containing 4ml 6% dextran in saline and 1% heparin so that final concentration of heparin is no greater than 25 units/ml. This mixture is then left at room temperature for approximately 30 minutes for the cells to sediment. The plasma containing the nucleated cells for culture is removed with a sterile Pasteur pipette. The cells are drawn through a 21g needle to separate any clumps of cells. The McCoy's 5A medium is then mixed in 9:1 concentration with autoclaved 3% agar. The bone marrow cells are added to this mixture to give a final concentration of 2 x 10^5 cells/ml and one ml aliquots pipetted on to the previously prepared underlays. After gelling at room temperature, the plates are incubated at 37°C in a fully humidified incubator with a constant flow of 7.5-10% CO_2 in air.

Colony formation in agar is critically dependent on the concentration of the agar and 0.3% is the optimal concentration, 0.4% being inhibitory for colony formation and 0.2% not gelling[5].

Colony counts are performed after seven and 12 days of incubation using a dissecting microscope. Colonies appear as aggregates of cells against a background of smaller aggregates of cells called clusters and the single cultured cells. A colony is arbitrarily defined as an aggregate containing more than 40 cells. A cluster contains 5-40 cells in an aggregate. Whilst the definition of a colony is an arbitrary one, it is based on recent studies done overseas[5].

Colony size depends on:

(i) concentration of "CSF"
(ii) number of cells initially plated
(iii) adequacy of media, foetal calf serum
(iv) efficiency of the CO_2 incubator in maintaining fully hydrated conditions at a constant pH.

All work is carried out in a laminar flow cabinet (Bassair — Sussex, England).

In order to study the cellular composition of the colonies, a finely drawn Pasteur pipette is used to pick the colonies out of the culture under the dissecting microscope. The cells are deposited on to a glass slide and then fixed in methanol for 10 minutes. The cells are stained with 0.6% orcein in 60% acetic acid for 10 minutes[2].

Results

(a) Cellular composition of colonies following 12 days' incubation — three main morphological types of colony have been observed.

(i) Aggregates with a dense central core of cells surrounded by a looser peripheral mantle of cells.

This type of colony usually consists of a central core of tightly packed granulocytes with the outer mantle composed of macrophages or monocytes (Figure 1).

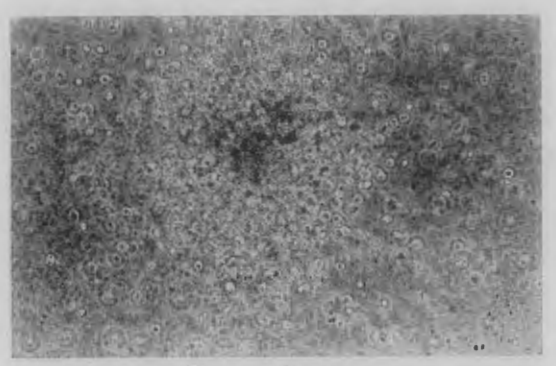

Figure 1. See text.

(ii) Loose aggregate of widely separated cells which are generally made up of macrophages (Figure 2).

(iii) Compact aggregates with no outer mantle of cells which consist of pure populations of granulocytes in varying stages of maturation.

Figure 2. See text.

In our experience the predominant colony is granulocytic, with a small population within the colony of monocyte-macrophages. The smaller more dense aggregates which are found largely at seven days' incubation, tend to be mononuclear showing a lesser degree of differentiation than the larger colonies.

In general, clusters are more numerous than colonies, this trend being more apparent at seven days of incubation (Table II). The

TABLE II

normal group consists of bone marrow aspirates from 26 patients in whom erythropoiesis and granulopoiesis were both active and normal, so that these serve as a control group. At seven days' incubation the mean cluster:colony ratio in the control group is 9.0, decreasing to 1.6 by 12 days' incubation.

At the time of culturing the normal group, marrow aspirates from six patients with acute myeloid leukaemia were also cultured (Table II), with a breakdown of these results shown in Table III. Generally, colony formation is poor with a greatly increased number of clusters forming per plate. A normal growth pattern is obtained when the patient is in remission.

A group of six patients diagnosed as having idiopathic thrombocytopenic purpura was also cultured. The bone marrow smears indicated normal and active erythropoiesis and granulopoiesis with abundant megakaryocytes. As can be seen from Tables II, IV, this group generally fail to produce the number of colonies which would be expected. Clusters were more numerous, giving a higher cluster to colony ratio than the control group. Further studies on these patients are required to clarify the reasons for the failure of normal colony growth.

TABLE III

TISSUE CULTURE RESULTS FROM PATIENTS WITH ACUTE MYELOID LEUKAEMIA

PATIENT	7 DAY CLUSTERS	7 DAY COLONIES	CL/CO	12 DAY CLUSTERS	12 DAY COLONIES	CL/CO
V.C.	3	0	3+	0	2	0.0
P.M. (r)	212	105	2.0	45	87	0.5
J.K. (r)	86	16	5.4	40	36	1.1
J.C.	56	4	14.0	8	1	8.0
M.L. (i)	2000	20	100.0	1120	84	13.3
(ii)	0	0	-	210	34	6.2
(iii)	44	13	3.4	12	13	0.9
R.G. (i)	295	0	295 +	53	2	26.5
(ii)	0	3	0.0	9	7	1.3
R.L. (i)	99	8	12.4	83	25	3.3
(ii)	0	0	-	7	1	7.0
(iii)	23	1	23.0	-	-	-

r = remission

TABLE IV

PATIENT	7 DAY CLUSTERS	7 DAY COLONIES	CL/CO	12 DAY CLUSTERS	12 DAY COLONIES	CL/CO
[illegible]	19	2	9.5+	-	-	-
[illegible]	58	21	2.8	-	-	-
[illegible]	21	0	21+	15	6	2.5
[illegible]	242	0	242+	280	7	40
[illegible]	0	0	-	25	1	25
[illegible]	106	0	106+	89	49	1.8

Discussion

This in vitro culture system allows an estimate of the progenitor cells in any haemopoietic tissue. This means that patients with acute myeloid leukaemia receiving chemo-

therapy can be monitored and the growth pattern assessed to establish response to treatment. A return to normal growth pattern in agar indicates that the marrow is recovering from the toxic effects of chemotherapy, and once remission is achieved, a completely normal pattern is obtained. An abnormal growth pattern signals relapse in these patients, with abnormal growth pattern preceding clinical relapse. With the patient P.M. Table III, a normal growth pattern was obtained in culture whilst the patient was in clinical remission. On examination of the colonies, we found that the colony was composed of mononuclear cells rather than more differentiated forms such as mature neutrophils. Twelve weeks later this patient was again studied and the growth pattern in the agar was abnormal, with only a few colonies per plate and a large number of clusters. This patient was also showing clinical relapse at this stage. The study of cellular morphology and differentiation is every bit as necessary as determining the cluster to colony ratio of each patient.

Peripheral blood from two patients with chronic myeloid leukaemia has also been cultured in agar. One patient showed a greatly increased number of colonies per plate with an increased number of clusters. These colonies showed a normal differentiation and maturation, with many mature neutrophils. Macrophages and monocytes were also found in some of these colonies.

A source of colony-stimulating factor is required in the plates throughout the culture period for colony formation. If no "CSF" is present, little or no colony formation occurs[10]. It has been suggested that this "CSF" represents a true granulopoietic regulatory substance[5]. The major source of "CSF" which stimulates human bone marrow has been peripheral white blood cells. Subsequent data has shown that the monocyte is the main source of "CSF" in human blood[3].

It is our hope that a prognostic value of the culture technique may be established for the evaluation of neutropenia of various aetiologies as well as measuring bone marrow proliferative capacity and to give some aid in monitoring chemotherapy in patients with acute myeloid leukaemia and related disorders.

Acknowledgments

We are indebted to Dr R. Hill and Mr A. Nixon for their continuous assistance and encouragement.

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Technical Communications

Positive Direct Coombs Test due to Sulphonylurea

Case History and Laboratory Results

An 80-year-old European woman, Mrs H., had a history suggestive of systemic lupus erythematosis, was a known diabetic and was on the sulphonylurea drugs chlorpropamide (in the form of diabinese) and tolbutamide to control her diabetes.

She was admitted to Taranaki Base Hospital in March, 1976, with a haemolytic anaemia of unknown origin. Her haemoglobin was 52g/litre and she had a positive quantitative direct Coombs test to a titre of 40, the immunoglobulin coating reacted with anti IgG, —IgM, —IgA, —C_3 and —C_4 (Dade monospecific Coombs reagents). All other investigations, including Ham's acid serum test and Donath-Landsteiner, were negative and it was not possible to show any specificity of the eluted antibody as it reacted only with Mrs H.'s own cells by routine methods. She

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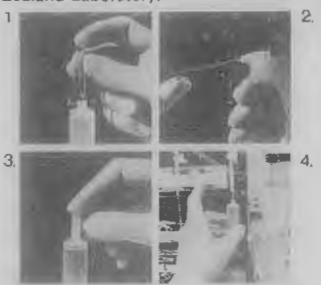

Instructions: 1. Puncture reservoir diaphragm with pointed polystyrene shield. 2 Make finger puncture. Allow blood to fill UNOPETTE capillary pipette. Carefully wipe excess blood from outside of pipette. 3. Seat pipette securely in reservoir. Gently squeeze reservoir several times to rinse all blood from pipette. Mix by inversion. 4 Insert aspirator tubing for pre-diluted samples in reservoir.

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was not transfused at this stage but was treated with steroids to which the anaemia responded rapidly and was discharged on 375mg/day chlorpropamide and a short course of prednisone before further investigations could be carried out. She continued in good health with her haemoglobin reaching a peak of 130 g/litre.

Mrs H. was readmitted later in November the same year in a glycosuric coma with a haemoglobin level of 95 g/litre, having dropped from 125 g/litre in one month. Her quantitative direct Coombs test was still positive, titre 640, although the immunoglobin coating was now IgG only. The Ham's acid serum test and Donath-Landsteiner were negative as before and L.E. cells were seen in buffy coat preparations. Her serum and eluate were tested against a fully typed commercial panel of cells by 4°C saline, Löw's Papain, LISS and indirect Coombs techniques but only reacted with her own cells.

A solution of diabinese was made by the method of Logue *et al*[3] (1930) by dissolving a crushed 250mg diabinese tablet in 0.15M sodium hydroxide and neutralising this with 0.15M hydrochloric acid and collecting the supernatant after centrifuging. This diabinese solution was incubated with Mrs H.'s serum, and with her eluate for 30 minutes at 37°C before being incubated with a panel of cells for a further 30 minutes, when it was washed three times and tested with Coombs reagent.

Fresh normal serum was tested in parallel and Mrs H.'s serum and eluate and the fresh normal serum was treated with the neutralised sodium hydroxide solution instead of the diabinese solution and similarly tested in parallel.

The results showed that Mrs H.'s serum and eluate sensitised all cells which had been incubated with the diabinese solution but did not react at all with untreated normal cells. Normal serum showed no reaction with treated cells, and Mrs H.'s serum and eluate and the normal serum which were incubated with the neutralised sodium hydroxide solution, similarly showed no reaction. One week after the patient was taken off the sulphonylurea drug she was clinically showing an improvement and her QDCT had dropped to a titre of 80. The patient died a week later due to uncontrollable atrial fibrillation.

This appears to be an example of a positive direct Coombs test due to the sulphonylurea drug, chlorpropamide. This drug, according to Garratty (1974)[2] is thought to act by the immune complex adsorption mechanism where it has been shown that some drugs (including the sulphonylureas) have a high affinity for their specific antibodies forming antigen-antibody complexes readily in the patient's plasma. These immune complexes become loosely bound to red cells non-specifically, often activating complement which may lead to intravascular haemolysis. This has been termed "The innocent bystander reaction" as it does not seem possible to demonstrate that the red cell takes any part in binding either drug or antibody.

Logue *et al* (1970)[3] and Bird *et al* (1972)[1] have both reported patients with drug induced haemolytic anaemia due to sulphonylurea and although it was not possible to show that Mrs H.'s haemolytic anaemia in March was due to sulphonylurea it was possible to show at a later stage that she had an autoantibody which sensitised cells only in the presence of the drug.

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New Plymouth.

January, 1977.

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Abstracts

Haematology

Evaluation of the Honeywell ACS 1000. Davis, A. E., and Steinbrink, C. F. (1977). *Amer. J. of Med. Technol.*, **43**, 20.

The Honeywell ACS 1000, an automated computerised scanning system, is evaluated against the recommended manual method for WBC differentials. Over 400,000 data points were gathered by both methods during an 11-month study. These data were found to exhibit an effective increase in throughput, precision, comparable accuracy, and a reduction in operator fatigue. —E. R. C.

Haemocytometry by Laser-beam Optics: Evaluation of the Haemac 630L. Lewis, S. M., and Bentley, S. A. (1977), *J. clin. Path.*, **30**, 54.

Hemac is an automated blood counting system which is based on production of impulses from light scattered and diffracted by cells flowing past a laser beam. The pulses are processed electronically for cell counts and packed cell volume; haemoglobin is measured as cyanmethaemoglobin; absolute values are computed. —E. R. C.

Microcytosis, Anisocytosis and the Red Cell Indices in Iron Deficiency. England, J. M., Ward, Susan M., and Down, M. C. (1976), *Br. J. of Haemat.*, **34**, 589.

Red cell volume distribution curves have been used to measure microcytosis and anisocytosis in normal subjects, blood donors and patients with iron deficiency anaemia. Three stages are suggested as iron deficiency progressively interferes with haemopoietic function. —E. R. C.

Impaired Anticoagulant Effect of Heparin in the Artificial Kidney. Bjornson, J., and Godal, H. C. (1976), *Scand. J. Clin. Lab. Invest.*, **36**, 581.

This study shows a rapid reduction in heparin anticoagulant effect (indicated by a marked shortening of the thrombin-clotting time) without a corresponding fall in heparin concentration (measured by polybrene titration) during dialysis of heparinised blood and plasma. —E. R. C.

Some Observations on the Preparation of Platelet-Rich Plasma. Woods, B. P., Dennehy, Angela, and Clarke, N. (1976), *Thrombos Haemostas (Stuttg.)*, **36**, 302.

In order to prepare platelet-rich plasma a constant height and volume of citrated blood was centrifuged at different gravity forces and times. Results are described showing that gravity force and time have an important influence on the platelet numbers and the plasma volumes recovered. —E. R. C.

Computerized Haematology. Operation of a High-Volume Haematology Laboratory. Drewinko, B., Wallace, B., Flores, C., Crawford, R. W., and Trujillo, J. M. (1977), *Am. J. clin. Pathol.*, **67**, 64.

This paper details the operation of a large Texas laboratory, on-line to a laboratory-dedicated computer. This computer stores, retrieves and monitors the results of two Coulter Model S cell counters, three Technicon platelet counters, one Electra 600-D, and six leucocyte differential consoles, which are interphased to the computer. All other haematology tests are batch-entered via the keyboard of cathoderay tubes. E. R. C.

Normotest-Thrombotest Discrepancy in Congenital Coagulation Disorders of the Prothrombin Complex and in Coumarin-treated Patients. Girolami, A., Brunetti, A., and Patrassi, G. M. (1977), *Am. J. clin. Pathol.*, **67**, 57.

A Normotest-Thrombotest discrepancy is claimed to reflect the presence of coumarin-induced inhibitors or intravascular coagulation, or both. The results of this study indicate, however, that a significant discrepancy is also present in all plasmas from patients who have congenital coagulation disorders of the prothrombin complex. —E. R. C.

An Automatic Leucocyte Analyzer. Validity of its Results. Arkin, C. F., Sherry, Maureen A., Gough, Arlene G., and Copeland, B. E. (1977), *Am. J. clin. Pathol.*, **67**, 159.

A statistical study was undertaken to evaluate the Corning Larc Leucocyte Analyzer by comparing its results with those of 20 medical technologists with regard to reproducibility and degree of agreement. This study indicated that the Larc showed at least as good a reproducibility as a group of well qualified technologists, and in many cases better; reported populations of different cell types (including abnormals) in proportions clinically equivalent to the manual method; and produced normal ranges similar to those currently in use. —E. R. C.

Purified Azure B as a Reticulocyte Stain. Marshall, P. N., Bentley, S. A., and Lewis, S. M. (1976), *J. clin. Path.*, **29**, 1060.

This paper compares reticulocyte preparations stained with several commercially available batches of brilliant cresyl blue, new methylene blue and purified azure B. The authors recommend purified azure B because it has the advantages of reproducibility and absence of dye deposits. They state that these advantages make the stain more convenient for routine use than those currently employed. —E. R. C.

An Improved Automated Method for the Measurement of Red Cell 2, 3-diphosphoglycerate. Purcell, Yvonne, and Brozovic, B. (1976), *J. clin. Path.*, **29**, 1064.

A modified automated clorimetric micromethod for the determination of red cell 2, 3-diphosphoglycerate (2, 3-DPG) is described. This method makes use of a Technicon Auto-Analyzer system, working at a rate of 30 samples per hour. —E. R. C.

Carl Wilhelm Scheele

a sad tale if ever there was one

CARL WILHELM SCHEELE
1742-1786

It is said of Scheele that his record as a discoverer of new chemical substances is probably unequalled. Besides discovering chlorine and ten important acids including citric, he prepared oxygen a couple of years before Priestley.

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Book Reviews

Cerebrospinal Fluid Cytology, An Introductory Atlas. 1st Ed. M. Oehmichen. Published by G. Thieme/W. B. Saunders, 1976. 208 pages, 263 figures, 12 colour plates. Price $NZ52.50. Obtained from N. M. Peryer Ltd., Christchurch.

As the title indicates this publication is intended to serve as an introduction and atlas for cerebrospinal fluid cytology. With the analysis of CSF by cytological methods becoming an acceptable and useful technique for diagnosis its publication is timely.

The obvious difficulty in obtaining specimens in central nervous system diseases demands that the best use is made of them. Information is given on cell concentration with step-by-step instructions. The rather slow sedimentation technique and more rapid filterisation techniques, including the use of millipore filters, are discussed.

The chapter on the anatomy and physiology of the CSF gives a succinct account of the origin of the cells and their differentiation illustrated with clear black and white photographs. Degenerative changes are also illustrated and criteria for distinguishing pathological cells provided. Other chapter headings inlude, Diseases and Cell Picture Alterations, Non-specific Irritative Processes, Metabolic Disorders and Brain Tumours.

While this may seem an expensive publication is does cater for a specialist area and covers new ground.

W. J. Stead.

Fundamentals of Clinical Haematology—4th Edition. Byrd Stuart Leavell. M.D., Oscar Andreas Thorup. M.D. W. B. Saunders Company, 1976. 755 pages, illustrated. Price $NZ30. Obtained from N. M. Peryer Ltd., Christchurch.

The authors intend this book to be a concise yet comprehensive volume on clinical haematology. Their pursuit of these contradictory goals is, I think, largely successful. The book is clearly set out and in the main easily read, with numerous references and an adequate index.

I do find difficulty in separating clinical and laboratory haematology and perhaps a defect in this book is the superficial descriptions of several commonly carried out laboratory investigative procedures. There is also a lack of precision in some of the sections on therapeutics.

This book, I think, would be most suitable for house physicians and registrars, but for the practising physician it will still be necessary to consult other texts in the management of several haematological disorders.

L. A. Bates.

Practical Clinical Biochemistry. Volume 2, Hormones, Vitamins, Drugs and Poisons. Harold Varley, Alan H. Gowenlock and Maurice Bell. 5th Edition 1976. Published by Heineman Medical Books Ltd., London. Obtained from N. M. Peryer Ltd., Christchurch. Price $NZ20.70.

"Varley" has always been an institution in the laboratory and a new edition after a lapse of nine years is something of an event.

This edition is divided into two volumes for easy handling and by chance the second volume has become available first. The first volume is still in the pipeline.

Thyroid function tests include all the current ones such as T_3 in various forms, T_4, TSH, stimulation and suppression tests using TRH (thyrotrophin releasing hormone) and thyroid hormone respectively. The older methods such as PBI and details of the BMR tests are still included. The steroid chapter contains new figures showing the steroids and their precursors and metabolites. The standard methods are described. The estimation of 11-deoxy-17-oxogenic steroids and the oxygenation index are covered. Tests used to elucidate diseases affecting the hypothalamic-Pituitary-adrenocortical axis are described and include insulin stimulation. Adrenocortical dysfunction is similarly dealt with and the protocol for hypofunction investigation suggests plasma cortical after Synachthen or actual ACTH estimation and in some cases the insulin stimulation test.

Tests of gonadal and feto-placental function cover about 90 pages. A great deal of this is discussion and explanation. However, the methodology is brought up to date. RIA and CPB (radio-isotopic counting techniques) are described for such things as LH and Testosterone. Function testing after LH/FSH releasing hormone or Clomiphene and their significance in gonadal function disorders and

infertility is discussed. Methods for oestrogens include that of Lever *et al* (1973), illustrated with the analyser manifold.

The chapters on the adrenal medulla and vitamins did not seem to provide much fresh information except a method for urinary methylmalonic acid. The level is related to B_{12} metabolism and is of importance in diagnosing the rare inborn error in infants.

The increasing significance of drug and poison detection and assay is reflected in the very long chapter on this topic, over 100 pages. Apart from drug assays the effect of drugs on other laboratory tests must be constantly kept in mind. The standard techniques of differential extraction for urine, gastric aspirates and blood, simple colour tests, chromatography by paper, TLC, GLC, spectrocolorimetry and infra-red spectroscopy are all described. Immunoenzyme assay did not apparently receive a mention and it may have been considered early days for this. Quantitative tests for barbiturates, amphetamines and anticonvulsants were mainly GLC. Hypnotics, benzodiazipines, chlorate and alcohol, spectrophotometric. Atomic absorption techniques are also used where applicable. Screening tests for phenothiazine and the tricyclic antidepressant are described.

This remains a very readable, practically orientated book and the traditional pattern of practical methodology and clinical explanation is an admirable feature when it is retained. I look forward to reading the first volume.

R. D. Allan.

Fundamentals of Clinical Chemistry, 2nd edition, 1976. Edited by Norbert W. Tietz. Multiple authors, 1,263 pages, illustrated. Price $NZ41.70. Published by W. B. Saunders Co., and obtained from N. M. Peryer Ltd., Christchurch.

Seven years have elapsed since the first edition was published and the increase in size from 983 pages to 1,263 pages and the altered emphasis reflects the development of clinical biochemistry in the intervening years. Quality control, chromatography, gas chromatography, thyroid function and vitamins are now allotted separate chapters and a chapter on computer systems is added. The authors read like a list of who's who in the clinical biochemistry world and it would be invidious to quote examples. In spite of this the style and content of the various chapters are not noticeably different.

There are many textbooks on clinical chemistry spanning the spectrum from the purely theoretical to the purely practical. Many are derived from or supplement courses in the subject at various educational institutions. "Tietz" has no such allegiance but is intended to provide the basis for teaching programmes in the subject. The material is therefore divided between the theoretical and the practical laboratory applications, followed by some interpretation in each chapter. It is comprehensive and provides an excellent coverage of basic biochemical mechanisms. This aspect does not change too quickly and remains useful for a reasonable time but instrumentation and technique changes with increasing rapidity and these topics tend to become dated. This occurred to me when examining the very full array of automated devices illustrated, which are already being superseded. The theoretical bases of instrumentation, the dynamics and mechanics of continuous flow automation, statistical information sufficient to calculate linear regression are all here for the examination candidate. Many practical techniques are provided but not always in great detail nor are automated options generally considered. Many more are simply alluded to. Practical application is not a strong feature of this book although application to the practical parts of the text is not unrewarding. It may be carping to single out apparent omissions in the wealth of information provided but I felt that the danger of hepatitis B infection merited more than a passing reference. Random checks failed to reveal any information about the significance of α_1 foetoprotein in amniotic fluid, the toxicology section did not provide a method for the common anticonvulsants and although RIA was mentioned EIA was not. The preface states that SI Units had been adopted in principle but in fact although comparable tables are provided in the appendix the text shows a rather distressing lack of conformity. Consistency would have been achieved by giving both units throughout.

This book is required reading for our medical technology examinations and is a good source book for that purpose.

R. D. Allan.

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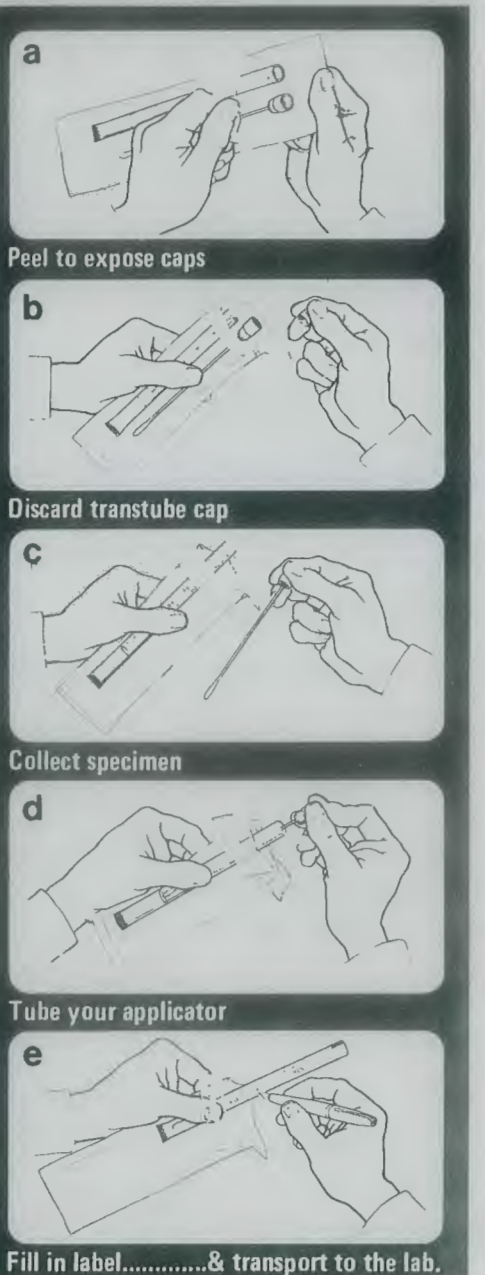

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Lynch's Medical Laboratory Technology, 3rd edition, 1976. Senior Author S. S. Raphael. In two volumes. Volume 1. 894 pages with 20 pages appendices, covers biochemistry and microbiology. Prize NZ$35. Volume II, 591 pages, covers histology, haematology and blood bank, cytogenetics and cytology. Price $NZ32.20. Both illustrated.

The earlier editions of this Canadian textbook entitled Medical Laboratory Technology and Clinical Pathology, came out in 1963 and 1969.

The third edition is dedicated to Dr M. J. Lynch, one of the original authors, who died in 1972.

The preface states that the authors' intention is to answer the question "how" and "why". It is designed mainly to provide medical technologists with the theoretical and technical information required for practical and teaching purposes.

The various sections have been independently reviewed.

Chemistry: 532 pages, 12 chapters. General chemistry and special urine tests have been deleted in this edition and automation and quality control added. Immunoglobulins appear in the microbiology section. The cell organisation and function is succinctly covered in some 30 pages and the illustrations are excellent. Laboratory organisation and safety now includes a description of the strict protocol required to avoid hepatitis infection. Quality control provides all the essential information for manual and automated systems and SI Units receive a mention. The significance of results and reporting was, I felt, rather neglected. Analytical systems discusses the principles of most laboratory instruments, but does not attempt to describe particular makes. Polarographic equipment, Nuclear Magnetic Resonance, Chromatograhy, Electrophoresis, radioisotope procedures and computers are included. A wide range of automated equipment is discussed and illustrated and the pros and cons of continuous flow, discrete systems and centrifugal techniques described. The remaining chapters deal with the various physiological systems, starting with anatomical and physiological considerations. There are quite a number of histological illustrations. General chemical pathology deals with tests which do not conveniently fall into the organ classification. Elementary toxicology, ultramicro analysis, aminoacids and inborn errors and endocrine investigations are other chapter headings. Enzymes are very fully discussed in a chapter of 48 pages. The physiological explanations would seem to cover most of the requirements of the NZIMLT examinations. Standard techniques were thoroughly covered. A few seemed a little out of date and there were some surprising omissions. With the explosive increase in medical technology it becomes increasingly difficult to achieve the ambition of confining all the essential information within the covers of one book and indeed two volumes are required in this instance. One wonders, in spite of the quantity of useful information garnered in these volumes, how valid the concept of the compendium is, in these days of specialisation. R. D. Allan.

The *Microbiology* Section, in 343 pages, does remarkably well to cover such a wide range of topics, from systematic bacteriology to medical entomology. Sterilisation is adequately covered. The culture media chapter is much improved, although there are some glaring omissions, for example XLD agar, Triple Sugar Iron agar. In this edition more emphasis is placed on rapid screening kits, e.g., Entero Tube, API.

Systematic bacteriology is compressed into 56 pages. At first glance this seems inadequate, but on further examination it is clear that much space has been saved by the use of tables and diagrams.

Mycology and parasitology (including medical entomology) are well done and well illustrated. There is a good introduction to virology and rickettsial disease which no doubt will be expanded in future editions.

Maree Johnstone.

Volume II, *Immunohaematology*: Three chapters. Principles of Immunohaematology, Principles of Blood Transfusion Therapy and Blood Bank Organisation and Methodology. A multidiscipline textbook has a limited appeal in a sectionalised laboratory. However, I can see that this type of book may be of some value to a small general hospital laboratory where only a few staff members would be carrying out all disciplines. To this end the chapters covering Immunohaematology and Blood Transfusion technique would perhaps be sufficient. In a specialised reference laboratory I can see no place for this book. It

may be used as a quick and easy reference but the potential students must be advised to further their knowledge by referring to the recognised standard reference books.

The text dealing with the blood group systems for example is limited to 14 pages and even so some of the text is rather dated, more information having been accumulated over the past year or so.

Generally the information is well presented and the techniques described are adequate. One glaring omission, however, is that related to the testing for Hepatitis. No mention is made of the second generation tests, e.g., passive haemagglutination, a technique currently more widely practised than radioimmunoassay, which is mentioned, a method limited in its application to more specialised centres. Similarly, the section dealing with blood preparation and component therapy; this would only be performed in more specialised centres.

Therefore if the book is designed for use in more specialised centres, as the techniques listed would seem to indicate, it would be quite inadequate as far as the information listed in regards to the blood group systems.

A. E. Knight.

Haematology: Contains six chapters covering Blood Cell Formation, Basic Techniques, Erythrocyte disorders, Disorders of Haemostasis and Investigation of Haemostatic defects. As one might expect in a multidiscipline volume of this type, the scope of the text is somewhat condensed. Nevertheless, all the information is up-to-date, concise and well tabulated with plenty of diagrams and photomicrographs of various cell abnormalities.

The methods covered in all sections are those currently in use, with photographs of modern equipment available for the performance of the various tests. The Haemostatic section contains useful pages with concise information on the coagulation factors which is not often found outside specialist texts.

This is a useful section for students in medical laboratory technology.

B. W. Main.

Cytogenetics: Three chapters of this book are devoted to Cytogenetics and related topics, the first chapter covering Basic Cytogenetics. This chapter provides a useful introduction for a newcomer to the field, covering topics such as cell division, classification and the normal human karyotype. Sex chromatins and the Lyon Hypothesis are very well presented at the conclusion of the chapter. I am, however, still searching for the ABO locus reference cited on page 1379!

The second chapter covers Clinical Cytogenetics, and is a useful reference section, particularly with respect to the sexual disorders. There is an adequate coverage of other commonly-encountered anomalies, with a good supply of references for further information.

The final chapter, entitled Laboratory Cytogenetics, gives techniques, as well as hints for the uninitiated. In the author's own words, "There are probably as many local modifications of the peripheral leukocyte culture technique as there are cytogenetics laboratories", and for this reason the chapter will be of limited value for most readers. A section such as this, however, would be useful for a laboratory expanding its activities or reappraising its techniques.

In summary, this volume would be a useful addition to a Cytogenetics library as it contains, in one volume, information which one usually has to glean from a variety of sources.

Wendy Chewings.

Cytology: 32 pages, one chapter. Despite the fact that seven years have passed since the last edition, there has been little revision of the text and no change in the illustrations, which is a pity, as there is room for improvement in several of the photographs. The author has rectified the omission of an explanation of dysplasia in this edition by supplementing the appropriate photographs with two brief paragraphs describing this condition.

A comprehensive account is given regarding obtaining of specimens, fixation, preparation and staining of smears for cytological diagnosis. I would query the statement that cells in pleural and peritoneal fluids degenerate very quickly. Quite the reverse happens if a delay occurs in processing these effusions. The cells are preserved and even multiply in the fluids which are good culture media.

My main criticism of the remainder of the chapter is the insufficient description given to normal and abnormal cells encountered in cervical smears and non-gynaecological speci-

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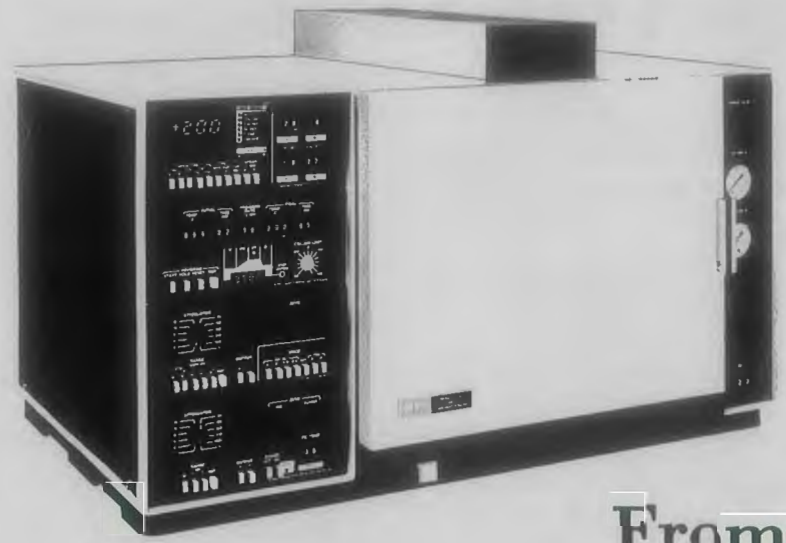

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(1) Klein, G. C. and Jones, W. L. : Applied Microbiol. 21 : 257, 1971.
(2) Janeff, J., Janeff, D., Taranta, A., & Cohen, H. : Lab. Med., 1971 (in press).

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mens. There is no illustration of a normal endometrial cell and words such as Karyorrhexis and squamous metaplasia are used but their meaning is not explained.

A detailed, in-depth account is given of hormonal vaginal cytology which because of its arrangement and repetitions is arduous to read.

Controversial points are made, such as the normal occurrence of basal cells in vaginal smears, the nuclei of Trichomonads staining red with Papanicolaou's stain and the differentiation between slight, moderate and severe dysplasia by just the *numbers* of abnormal cells in a smear.

Detection of malignant cells is the most important aspect of most Cytology Laboratories and this should be emphasised in all textbooks dealing with the subject. My impression is that this Cytology section falls short of these criteria. Brenda E. Brown.

Histology: Since the death of Dr Lynch, the Histology section of this composite book has become the responsibility of C. F. A. Culling. The change of author has resulted in very little change in the text; on first perusal the third edition seems identical to the 1969 publication.

Careful reading reveals some expansion in the sections on Carbohydrates and Lipids and the occasional deletion or addition of methods tested by time. The 185 pages are well packed with a remarkable amount of information, ranging from the action of fixatives to the identification of cerebral lipids. All the major subjects are covered, including a little on electron microscope preparations and histochemistry. As a textbook of histological technique this work can almost stand by itself, but it is aimed at the senior technologist at a Canadian "Community Hospital Laboratory" and is not suitable for the inexperienced. The photographs inserted in the text are in black and white and are sometimes helpful, but there is no consistent attempt to show the results of staining methods. Perhaps the colour filmstrip offered by the publisher would help.

It is pleasing to find clinical information and discussion amongst the methods given, and this does make the presentation more interesting.

This is the first time that this composite book has appeared in two volumes. Perhaps next time it will be printed in at least four parts, then there will be a place for one on the Histology bench. B. Glynn-Jones.

Bacteriology Illustrated, R. R. Gillies and T. C. Dodds, 4th Edition, 1976. 260 pages. Published by Churchill Livingstone and obtained from N. M. Peryer Ltd., Christchurch. Price $NZ17.60.

Although not inexpensixe, this colour atlas has much to recommend it, particularly the faithful reproduction of colonial morphology in relation to culture medium. Clearly it cannot examine the pathogen in any depth, but an honest attempt has been made to put the most common isolates into clinical perspective. The chapter on Bacterioides may be considered over-simplified. At the same time the Lactobacilli seems to have pride of place in one of the new chapters.

This little book will be more suited to the medical student than the laboratory technologist, particularly where the notes deal with specific pathogens associated with a particular disease process. Additionally, some of the more recent technical advances have their place in the flow charts and text.

H. Shott.

Biomedical Applications. Volume I, Number 1, 116 pages.

This is a new publication of the Elsevier Scientific Publishing Company, P.O. Box 330, Amsterdam. It is an integral part of the Journal of Chromatography but can be subscribed to separately. Biomedical Applications will appear six times a year. Subscription approximately $US50. A sample copy may be obtained on request. The first issue contains articles on metabolic patterns in maple syrup disease by means of gas chromatography and mass spectrometry, quantitative gas chromatography and single -ion detection of aliphatic -keto acids from urine, tryptophan and kynurenine determination, microchromatography of haemoglobins, chromatography of sugars in body fluids, study of serum cholinesterase variants, micromethod by gas chromatography of serum theophylline, rapid TLC for carbazepine, diphenylhydantoin, mephenytoin, phenobarbital and primidone in serum, TLC for digotoxin in serum, identification of an interfering compound in the GLC determination of N^2, N^2-dimethylguanosine, improved radioimmunoelectro-

phoresic assay of serum thyroxine-binding globulin, TLC for paracetomol and a review of simple lipids and their constituents by routine chromatography. R. D. A.

Microbiology — 1976. Edited by David Schlessinger. Published by American Society for Microbiology, Washington D.C., 1976. 587 pages.

Microbiology — 1976 is a three-part volume comprising papers presented at ASM conferences in 1975.

Part I, the largest section, 449 pages, is entitled Bacilli Biochemical Genetics, Physiology and Industrial Applications. In this section 47 papers from the ASM conference on Bacilli are presented. The much-studied *Bacillus subtilis* seems to be the organism of choice. Most papers deal with the biochemical genetics and physiology of this organism, studies on bacteriophage featuring largely. Perhaps the paper of most interest to medical microbiologists would be " Aminoglycoside Antibiotics Produced by the Genus Bacillus ". Here the antibiotic butirosin produced by some strains of *B. circulans* is compared with other aminoglycosides.

Part II comprises four papers on *Neisseria gonorrhoeae*. This section is more medically orientated and includes a paper on the antibiotic resistance of gonococci and an interesting paper on this organism's evolution traces the emergence of sulphonamide resistant strains of the 1940s to today's problem of increased penicillin resistance.

The third chapter, entitled Genetics and Molecular Biology of Industrial Microorganisms outline the types of organisms of major industrial importance, mainly those concerned with the synthesis of antibiotics. Other topics of interest are " The Mycoviruses, their Significance in Industrially and Agriculturally Important Fungi ", and a paper on the substance that is produced by *B. thuringiensis* which is useful in the control of insect pests.

While this is not a book to be found on the hospital laboratory bookshelf, out of a total of 76 papers presented there is surely something of interest to be found by everyone in the microbiological field.

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NEWSLETTER

An official publication of the N.Z. Institute of Medical Laboratory Technology (Inc.)

Vol. 9, No. 4 July, 1977

Council Notes

A Council meeting was held in Christchurch Hospital on May 5 and 6, 1977, Mr B. W. Main in the chair. Council also took the opportunity to discuss relevant matters with Mr N. McDougall who was there for a SHEO hospital committee meeting.

CONDITIONS OF EMPLOYMENT

Grading Committee: Council discussed the points raised in a letter from the Auckland Branch and decided to make further enquiries about our submission to the Board of Health Enquiry into the Clinical and Public Health Laboratory Services relating to the functions of the Grading Committee. We saw these as setting the maximum grades for posts and allowing the local hopital board to determine the starting point with the right of appeal in the event of dissatisfaction on the part of the applicant.

The Grading Committee is scheduled to meet some time in July but the reimposition or extension of the wage freeze is likely to impose the same restraints as last year.

Negotiations: There is no progress to report regarding the salary claim but negotiations are still scheduled to take place. We will be considering a loss of comparability and also laboratory assistants' salaries. In the meantime the Tribunal chairman has given a decision on the "half-yearly" 3.5 percent increase.

A part of this "package deal" is to restore margins. The basis of this is the assumption that the salaries now greater than $6,000 should have increased by 100 percent between April 1970 and January 1976. Salaries would be increased from May 15, 1977 by the percentage required to give a 100 percent increase on these specified dates.

SHEO: Mr J. Elliot of Wellington has been appointed SHEO and CSSO representative to replace Mr D. Bolitho who has succeeded Mr H. E. Hutchings as Head of the Science Department at The Central Institute of Technology. Some discussion has taken place regarding a change of name to that of Health Service Employees Organisation. The implication is that a wider range of employees could be catered for.

Vincent Hospital Board: The Ombudsman has indicated verbally to Mr Morris that he is unable to upset the Board's decision other than to ask them to continue his employment until 30/6/77. It is proposed that SHEO look at the implications when a copy of the Ombudsman report is received.

Wanganui: No developments had taken place since the Hospital Board had asked the Health Department to set up a one-man commission of enquiry. SHEO had made known its concern in this case.

EDUCATION

Coagulation Workshop: Plans had been made to hold this at Christchurch on Monday and Tuesday, May 9 and 10, following the seminar at the weekend. Seven of the 12 laboratories invited to send technologists had done so and others attended at their own expense.

Technical Assistants: The examinations took place on May 10 and 11. There were 139 candidates for QTA, 9 for QTO, 43 different examination papers had to be prepared and 33 examiners were involved.

TCA: Mr A. E. Harper has replaced Mr H. R. Hutchings as MLT representative. The secretary of the Auckland Branch wrote to Council seeking help in expediting examination results from the TCA. It was stated that Auckland Hospital Board required official notification of results before salary increases were sanctioned. This is a long-standing problem and relates to our unfavourable place in the computer queue. Council thought that in view of the circumstances the initial advice of examination success should be sufficient and proposed to communicate this view to the Auckland Hospital Board and the Director of the Auckland Hospital School of Technology, Dr M. Gill.

Fellowship: Further to alternative routes for obtaining Fellowship, Council agreed that published work should be accepted as a basis for Fellowship. Candidates may submit copies of significant work published, to the Fellowship Sub-committee. It would then be submitted to two or more assessors for their opinion. The work would have to be of a substantial nature and if jointly published work was submitted it would be necessary to show that the candidate had made a major contribution to it.

MTB: Amendments to the regulations in reference to Board membership have been forwarded to the Law Drafting Office.

The Seal and Certificates are in the hands of the Government Printer.

Council were invited to reconsider their view that the QTO examination should be discontinued and that an exception be made in the case of certain groups but Council adhered to the view that the examination should be discontinued after 1980 which provided time for alternative arrangements in training and qualification to be established.

A joint MTB, Technical Institute Workshop was held at the Central Institute of Technology, March 15 to 17. 21 persons including medical technologists, part-time and full-time tutors from the technical institutes, a representative from the Auckland Medical School and a representative from the CIT Pharmacy School attended. Good progress was made and agreement reached. The basic subject headings of laboratory practice, anatomy and physiology and clinical chemistry were retained and a further subject, human relations, added.

The first full-time year was divided into basic blocks of learning of approximately equal hours. thus: 24-26 hours a week, 6 weeks to compose one module of learning, 2 modules, one unit or term with three terms in one year. It was recommended that the concept of the diploma be reaffirmed and that discussion with relevant educational bodies be arranged and that a further meeting be held to extend the work already done and to consider years 2, 3 and 4.

The MLB has now approved aegrotat passes for Part II and III examinations.

MISCELLANEOUS

Awards: The decision of Roche Products (N.Z.) Ltd to donate the triennial awards in Microbiology and Clinical Biochemistry was noted with satisfaction.

Efforts are being made to organise a biennial award to help a suitable candidate attend and represent the Institute at the IAMLT Biennial Congress. It was envisaged that this would be a joint effort and that the Institute would contribute to this.

Standards Sub-committee. A meeting of the steering committee compiled of two representatives from the three professional organisations is to take place at the National Health Institute in Wellington 16/5/77. The intention is to establish a permanent committee and seek official status. Working sub-committees to review and organise procedures for the various specialities are envisaged. Standardisation and quality control is well organised in certain areas and a great deal of relevant data is available.

Publications: The Journal and Newsletter have been severely pruned this past year to effect economies and Council recommended that sufficient money be made available to support the essential functions of the publications, namely to provide a focus and identity for the Institute and to disseminate relevant information.

Finance: The Treasurer reported that it would be advisable to propose an increase in subscriptions this year to insure a balanced budget. Inflationary effects could not be ignored and essential services such as adequate meetings of the negotiating and other sub-committees, sufficient finance for the publications and secretarial help, which is minimal, should not be jeopardised for lack of a modest increase in subscriptions.

Rules: Concern was expressed at the allegation that some branch officers were not members of the Institute. It was noted that Rule 29 required all branch members to be financial members of the Institute and that the Branch Secretary submit a roll of branch members to the Institute Secretary. The other requirements should be noted. The Council requires all branch secretaries to make themselves known to the Institute Secretary in the first instance so that communication can be established.

Management: The sub-committee report noted the resignation of Mr R. McKenzie and it was agreed that Mr G. George be invited to replace him. Correspondence on the Hospital Central Purchasing System revealed some dissatisfaction with the service provided. It appeared that there was a need for a wider spectrum of medical technologists to assess products before bulk purchasing was done. Council referred the matter to the sub-committee for firm proposals. A letter from Mr K. Ronalds stressed the advantages of standard conditions of appointment for charge technologists and made suggestions along these lines. The sub-committee were asked to consider these proposals. Council also agreed that a meeting with Professor Hines at Massey University to discuss the Diploma in Health Administration and its relevance to our occupation would be useful and suggested that the local representative might undertake this.

Leave of Absence: The Secretary has been granted leave of absence from May 30 until August 6. His duties will be taken over by Mr K. McLoughlin.

Conference: Queenstown, 1977. Chairmen and guest speakers: General Forum, Mr D. J. Philip; Clinical Biochemistry, Professor I. G. T. Sneyd; Haematology, Dr A. E. White; Immunohaematology, Mr K. McLoughlin; Microbiology, Mr M. McCarthy: Small Laboratories, Mr M. Ford; Management, Mr R. Smaill; Dunedin Seminar, Professor J. B. Howie.

The guest speakers are Mr M. G. Garratty, FIMLS, Haematology/Immunohaematology, Research Associate, San Fransisco and Mr J. R. A. Hughes, Senior Lecturer, School of Business Administration, University of Otago.

The 1979 Conference will be held in the Auckland Medical School, August 15-17.

Applications for Membership

Mr S. J. Arthurs	Auckland	GT
Miss L. E. Bescke	Tauranga	TR
Mr A. M. Buchan	Dunedin	TR
Mr D. J. H. Burt	Palmerston North	GT
Miss A. R. Butt	Auckland	TR
Mrs L. E. Caulfield	Auckland	TO
Miss D. F. Collins	Dunedin	LA
Miss G. Cuthbert	Auckland	TR
Miss A. P. Dervan	Auckland	TR
Mrs J. Fraser	Invercargill	TA
Miss V. J. Grant	Hamilton	TR
Miss B. E. Haley	Auckland	TR
Miss W. M. Harris	Wellington	TR
Mr D. J. Hebden	Palmerston North	GT
Miss R. M. Holmes	Dunedin	TR
Mrs C. K. Jacobsen	Lower Hutt	AS
Miss C. L. Julius	Lower Hutt	LA
Miss M. L. Kennedy	Gisborne	LA
Ms J. A. McLean	Dunedin	LA
Miss J. A. McKnight	Dunedin	LA
Miss R. D. Maskelyne	New Plymouth	LA
Miss R. E. Muschamp	Dunedin	TR
Miss S. L. Newell	Dunedin	TR
Miss A. J. Nicoll	Gisborne	TR
Miss G. J. Parslow	Dunedin	TR
Mr D. A. Paterson	Tauranga	TR
Miss R. A. Reeve	New Plymouth	TR

Mrs A. M. Rush	Gisborne	GT
Miss V. J. Sadgrove	Wellington	LA
Miss J. E. Scheib	Napier	TR
Miss J. Shue	Lower Hutt	TR
Mr B. I. Shand	Dunedin	GT
Miss M. A. Smith	Auckland	BTC
Miss J. M. Stacey	Auckland	BTC
Mr D. V. Todd	Tokoroa	BTC
Miss J. M. Wall	Dunedin	LA
Miss V. J. Wallace	Dunedin	TR
Miss K. J. Williamson	Dunedin	LA
Mr J. R. Wilkins	Wellington	LA

Applications for Associateship

Mr S. J. Arthurs	Auckland	1975
Sandra Mary Bailie	Christchurch	1976
Judith Anne Eaton	Christchurch	1976
Trevor Noel English	Christchurch	1976
Gary Steven Milicich	Blenheim	1971
Janet Mary Montgomery	Christchurch	1976
Jacqueline Marie Muschamp	Palmerston North (now overseas)	1976
Gloria Ellen Sullivan	Christchurch	1976
Jacqueline Ilene Young	Christchurch	1976

Resignations

Miss M. A. Cooper	Auckland	LA
Mrs G. Cormack	Kaitaia	AS
Mrs S. K. Forbes	Wellington	QM
Miss J. E. Fraser	Gore	LA
Mr J. M. Kim	Masterton	AS
Mrs J. Macky	Auckland	P1
Mrs K. D. Miles	Rangiora	LA
Miss J. Rea	Napier	TAV
Miss J. E. Thomas	Wellington	P2
Mr D. Wilson	Auckland	AS
Mrs I. Wallmannsberger	Tauranga	AS

BRANCH NEWS

The South Island Seminar was held at Christchurch Clinical School on Saturday, May 7. Over 100 people attended. Several guest speakers discoursed on topics of general interest. Dr M. E. J. Beard on bone marrow transplant; Dr M. Fahey on Death on the Roads: and Dr W. M. Platts on the Diagnosis of Gonorrhoea. Institute affairs were also discussed and the SHEO executive officer addressed the meeting.

The Wellington Branch is organising a seminar on June 25.

The Dunedin Branch discussed remits 24.5.77 and formulated a remit to the effect that persons undertaking 20 or more days of weekend duty each year should be entitled to four weeks leave without prejudice to the existing regulations.

SHEO Newsletter. SHEO held its Annual Conference, 25.3.77. Details of claims settled or currently being programmed were given. The President, Mr A. J. Clarke and the Executive Officer, Mr N. McDougall are to commence a complete tour of all SHEO Hospital committees. Prior notice will be given.

The Commonwealth Foundation. Occasional paper No. XL has been circulated to the constituent members. The Commonwealth Foundation was established to administer a fund for interchanges between organisations in professional fields throughout the Commonwealth. This paper entitled "Problems Facing the Medical Laboratory Profession within the Commonwealth," is a report on a seminar convened in London, January 1976, by the IMLS. Stimulated by the shortage of fully trained medical laboratory scientists and equipment in many areas the Foundation voted a general purpose grant of £10,000 in 1972 for commissioning an analysis of existing gaps and the report reflects the main points emerging from an analysis of the data collected. There are regional zones, New Zealand belonging to a large group of eastern countries. Mr D. J. Philip represents the NZIMLT in this geographical group.

THE INTERNATIONAL ASSOCIATION OF MEDICAL LABORATORY TECHNOLOGISTS

A limited number of copies of the IAMLT magazine, "Med Tec International" was received and has been distributed as equitably as possible through the regional representatives.

It contained an illustrated account of the 1976 Congress and reproduced three papers read at the Congress. These were, Parasitology in Cytological Practise, Plague Surveillance in Southern Africa and the Histological Demonstration of Tyzzer's Disease. Other items were IAMLT Awards, list of addresses of 29 Member Societies, The Medical Laboratory in the Next Five Years and News from the Societies. In the Netherlands a wide range of paramedical staff have concluded a collective agreement regulating their legal status and conditions of employment. In Ireland final arrangements have been made to introduce a two year full-time course and a one year "in service" training course for certification in medical laboratory science. A university course in medical laboratory technology has been established in South Africa conferring a B.Sc. (Pathology).

Three medical laboratory associations in Germany united to form a body with the initials DVTA seven years ago and they are striving to improve their conditions. State registration is obligatory. A journal has been started but, "it is a hard and difficult task to find articles of interest and papers from our colleagues." (It's the same the whole world over!—Ed.)

Council discussed the need for active participation in the IAMLT and ways and means of ensuring representation at the next Congress in Edinburgh, 1978.

The editor undertook to write an article on the NZIMLT for the next issue of Med Tec International.

A series called Symbols of the Societies in the current issue features the insignia of the NZIMLT.

PUBLICATIONS REPORT

The observant will note that the July Journal only contains Haematology Abstracts. There are two reasons for this: firstly, the Editor had granted himself a month's leave and had to adhere to the scheduled production dates which eliminated late copy. However, there would not have been any Microbiological Abstracts *for lack of a volunteer to produce them.* This is not a hard task for someone accustomed to read the current journals and imbued with a modicum of professional zeal. One volunteer, fall out smartly please!

The schedule for the November Journal calls for the bulk of material to be submitted to the printer in early September. This is too late for Conference papers after the event but there is no reason why they should not be presented *before* the Conference. Run off a copy and send me the original now! You could be on the way to a Fellowship! (See Council Notes.)

Textbooks seem to be strangely inadequate for teaching purposes and tutors prepare their own versions of topics being taught.

Presentations of basic topics properly researched and referenced for teaching purposes would be invaluable for tutors and trainees generally. A series of tidy teaching notes would be a worthwhile project for the Journal and I would be grateful for contributions from those engaged in instructing the young. I am sometimes told by way of encouragement the gratifying news that the young show a disinclination to read the Journal and something of an educational nature directed at them might encourage them to do so. In the meantime my advice to the custodians of the young is to question them closely on the contents and if they don't know the answers, furl up the unopened Journal and clip them sharply round the ears with it! All joking apart if the contents of the Journal are not to your liking make a positive contribution by sending some material, it is *your* Journal.

LABORATORY WORKERS REGULATIONS

In order that members of the Institute appreciate their entitlement under their working regulations I have summarized the Laboratory Workers Regulations (DG 19) and the Standard Conditions of Employment (DG 48) which apply to staff (other than medical or scientific officers) employed in public hospital or Department of Health laboratories.

Copies of the full regulations are held in your Board office and by your local Council member. Under no circumstances should the summarized regulations below be used when discussing conditions of employment with your Board office.

1. **Hours of Work** (DG 19):
   Ordinary hours are 8 per day on 5 consecutive days.
2. **Meal Periods and Rest Breaks** (DG 48):
   All employees must have a meal break of at least ½ hour after 5 hours work. If not the meal period shall be taken as part of work time. Staff are entitled to morning tea, afternoon tea and supper breaks of 10 minutes for which free tea, coffee, milk and sugar must be provided or an allowance paid of 27 cents a week.
3. **Overtime and Penal Time** (DG 48):
   Conditions: Staff called back to work outside ordinary hours shall also be paid for travelling time. Minimum for call back is 2 hours, except an employee cannot be paid for the same hours twice. Staff must have 9 consecutive hours off-duty between ordinary hours of work.
   Rates—Overtime: 1. Between midnight Sunday and noon Saturday the rate is T1½ for the first 3 hours and T2 thereafter except overtime worked between 10 p.m. and 6 a.m. which is at T2. 2. Between noon Saturday and midnight Sunday all overtime is at T2.
   Penal Time: 1. Between midnight Friday and noon Saturday at T½ in addition to ordinary pay. 2. Between noon Saturday and midnight Sunday and on public holidays at T1 in addition to ordinary pay.
   Night Rate: Normal hours worked between 8 p.m. and 6 a.m. shall be at T¼ in addition to ordinary pay.
4. **Holidays** (DG 48):

A. *Public Holidays*: These are New Year's Day, N.Z. Day, Good Friday, Easter Monday, Anzac Day, Queen's Birthday, Labour Day, Christmas Day, Boxing Day and your local Anniversary Day. If any public holiday (other than N.Z. Day and Anzac Day) fall on a Saturday or Sunday, then the next succeeding day shall be taken.
If an employee is required to work on a public holiday he may—
1. Be paid T1 in addition to normal pay and have equivalent time off.
or 2. Be paid at T2 in addition to normal pay.

B. *State Service Holidays*: These are two holidays prescribed by the Board (usually December 27 and January 2). If an employee is required to work on a State Service Holiday no penal rate is paid but the employee is granted equivalent time off. Overtime (T2) is payable only if more than 8 hours is worked on a State Service Holiday.

C. *Recreation Day*: After being employed for a year an employee is entitled to one day (or two half days) recreation leave. It must be taken each year or is forfeited.

5. **Annual Leave** (DG 48):
   Under 10 years service—15 working days. Over 10 years service—20 working days.
6. **Sick Leave** (DG 48):
   Sick leave is granted on account of sickness not arising in the course of employment (this is classified as an accident and covered by the Accident Compensation Act).
   A schedule (DG 48 6 (2)) prescribes sick leave entitlement but during periods off sick this is reduced by the number of days sick including weekends and holidays.
   Under special circumstances an employee may take sick leave to attend to his wife or sick children (DG 48 6 (4)).
7. **Special Leave** (DG 48):
   These include bereavement, maternity and jury service leave. Reference should be made to the full regulations for entitlement and conditions.
8. **Allowances** (DG 48):
   Adult Allowance: A 20-year-old shall receive an allowance to bring his salary up to $4,739 (from 14.3.77).
   Married Allowance: An employee supporting a wife, husband or child shall receive an allowance to bring her/his salary up to $4,625 (i.e. the employee would have to be under 20 to qualify).
   On Call Allowance: The rates are—
   Monday to Friday: 7 a.m. to 5 p.m., 30.0c an hour; 5 p.m. to midnight, 96.1c for period; midnight to 7 a.m., 48.0c for period.
   Saturday, Sunday and public holidays: 7 a.m. to 5 p.m., 39.5c an hour; 5 p.m. to midnight, 96.1c for period; midnight to 7 a.m., 48.0c for period.

However if there are less than four people capable of being on call the following allowance is paid: one available employee, $350 a year; two available employees, $195 a year each; three available employees, $153 a year each.

Transport Allowance: If an employee is called back to work he shall be reimbursed actual and reasonable travelling expenses (i.e. a taxi) (DG 19).

A small transport allowance is also payable to staff required to travel to work at a time when no public transport is available which would be available during normal working hours (see DG 48 8 (6)).

Higher Duties Allowance: This is payable (subject to certain conditions) to a qualified technologist who is performing the duties of a position higher than his own (DG 48 8 (7)).

Other allowances include lodging allowance (8 (3)) and Remote Locality Allowances (8 (4)).

9. **Miscellaneous Provision:**
Protective Clothing — to be provided and laundered free by the Board (DG 48).
Refund of Annual Practising Certificate Fee — Registered Medical Technologists can have fees refunded by the Board (DG 48).
Fees for Lectures—Qualified staff giving a one hour lecture out of hours shall be paid $2.50 (DG 19).

B. T. EDWARDS,
Secretary NZIMLT.

MEDICAL TECHNOLOGISTS' BOARD CERTIFICATE OF PROFICIENCY IN MEDICAL LABORATORY TECHNOLOGY
SINGLE SUBJECT EXAMINATION— OCTOBER 1976
THEORY EXAMINATION HAEMATOLOGY PART III
PAPER I
(short answers)

INSTRUCTIONS:
1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of thirteen questions.
3. ALL questions are to be attempted.
4. Mark distribution for each question is shown. *N.B.* The total of marks is *150*.

1. Write notes on the application of any four radioisotopes used in haematology. (4 marks)
2. Write notes to show that you understand clearly the uses and limitations of the following in haemostatic investigation.
(a) Protamine Sulphate; (b) Epsilon Aminocaproic acid; (c) Stypven; (d) Ristocetin; (e) Reptilase; (f) Trasylol; (g) Russell Viper Venom; (h) Arvin; (i) Ancrod; (j) British Standard Thromboplastin. (20 marks)
3. Name the common synonym for the following:
(a) Cooley's Anaemia; (b) Haemophilia B; (c) Vaquez-Osler Disease; (d) Lederer's Anaemia; (e) Marchiafava-Micheli Syndrome; (f) Werlhof's Disease; (g) Osler-Rendu-Weber Disease; (h) Osteosclerosis; (i) Erythroleukaemia; (j) Familial Acholuric Jaundice. (10 marks)
4. Discuss any four special cytochemical stains you have used in diagnostic haematology giving:
(a) The uses and limitations; (b) The *principle* of the method used. (8 marks)
5. Write notes to show that you understand clearly the distinctive features of any four inherited cytoplasmic variations in leucocytes as would be shown in a peripheral blood film. (8 marks)
6. Write notes on the normally expected peripheral blood film appearance of the following haemoglobinopathies:
(a) Sickle Cell Anaemia; (b) Haemoglobin C Disease; (c) Beta-Thalassaemia Major; (d) Beta-Thalassaemia Minor; (e) Haemoglobin H Disease. (10 marks)
7. At a pH of 8.6 on Cellulose Acetate arrange the following haemoglobins in order of movement *toward* the anode in haemoglobin elecrophoresis.
F; A; A_2; D; C; H; S; M. (8 marks)
8. Write brief notes on the following:
(a) Sezary Cells; (b) T-Cell Acute Lymphocytic Leukaemia; (c) Denver Classification; (d) Unstable Haemoglobins; (e) McLeod Phenotype; (f) Immunoglobulin; (g) Streptokinase; (h) Niemann-Pick Disease. (16 marks)
9. (a) What are the most common causes of haemoglobinuria?
(b) Discuss the tests available for use in the differentiation of the chronic haemoglobinurias. (12 marks)
10. List the three most common hereditary red cell enzymopathies stating:
(a) Their mode of inheritance; (b) Where they occur in the glycolytic metabolic pathway; (c) Their respective peripheral blood film appearance; (d) Any further tests which may be helpful in their differentiation. (12 marks)
11. List the normally expected findings in a bone marrow aspirate of the following conditions, commenting in each example on the three cellular elements of the marrow and also on the expected iron stain result.
(a) Myeloma; (b) Megaloblastic Anaemia; (c) Chronic Granulocytic Leukaemia; (d) Iron Deficiency Anaemia; (e) Acute Acquired Haemolytic Anaemia. (15 marks)
12. (a) Name two drugs which are used in the treatment of Chronic Granulocytic Leukaemia; (b) Name four drugs which are known to cause changes in erythrocyte surface membrane; (c) What changes can occur in erythrocyte or leucocyte morphology in association with alcoholism?; (d) What information may a red cell folate estimation give in comparison with a serum folate estimation? (12 marks)
13. Discuss the differentiation of primary and secondary polycythaemia under the following headings:
(a) The peripheral blood film appearance; (b) Bone marrow findings; (c) Other tests which may be useful in differentiation. (15 marks)

PAPER II

INSTRUCTIONS:
1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.

2. The paper consists of seven questions. Question 1 is compulsory and *FOUR* other questions are to be attempted.
3. All questions carry equal marks.

1. Describe in a few sentences the major contributions of the following to haematology:
(a) Thomas Bothwell; (b) William Crosby; (c) John Dacie; (d) William Dameshek; (e) Paul Ehrlich: (f) Herman Lehmann; (g) Judith Pool; (h) Oscar Ratnoff; (i) Karl Vierordt; (j) Rudolf Virchow.
2. Describe the advantages afforded modern diagnostic haematology by scanning electron microscopy.
3. Write an essay on the use of radioactive isotopes in diagnostic haematology.
4. Illustrate the concept of "hypersplenism" with reference to the acquired haemolytic anaemias.
5. Discuss the haematological diagnosis of parasitic infestations found in the Pacific Basin.
6. Describe the application of cytogenetics to the study of the human leukaemias.
7. Critically assess the laboratory detection of female carriers of the genes for classical haemophilia and Christmas disease.

PRACTICAL EXAMINATION
HAEMATOLOGY PART III

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper and familiarisation with equipment, etc. At the end of this time, fifteen minutes extra is allowed for writing up only. NO technical work may be done in this time.
2. ALL questions are to be attempted.
3. The paper consists of five questions.
4. Mark distribution is shown for each question.

1. You are provided with peripheral blood films (1-7) and bone marrow smears (8-10). No differential counts are required. Brief clinical particulars are provided on Annex A.
(a) Comment *fully* on your findings; (b) Where possible a presumptive diagnosis should be given. (50 marks)
2. Four electrophoretic strips are provided. These are haemolysates of human blood run on cellulose acetate at pH 8.9 for 30 minutes and stained with Amido Black. A normal strip is provided for comparison.
Examine these and comment fully on your impressions. (8 marks)
3. Four photographs of human karotypes are provided.
Examine these and comment on your impressions. (10 marks)
4. Two test plasma samples A and B and a normal plasma C are provided.
(a) Carry out a Factor XIII screen test on the plasmas and comment on your results; (b) What further tests or investigation would you suggest be carried out?
Reagents will be supplied on request. (16 marks)
5. Two bone marrow slides **X** and **Y** and a positive control film Z are provided.
Perform a Perl's Iron Stain on the slides, and comment *fully* on your findings.
Reagents will be supplied on request. (16 marks)

THEORY EXAMINATION
HISTOLOGY PART III
PAPER I

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of five questions.
3. Attempt THREE questions only.
4. All questions carry equal marks.

1. Discuss microtome knife sharpening and the various techniques and machines available.
2. Compare and contrast methods used for the decalcification of tissues.
3. Discuss the principles of fluorescent microscopy, the apparatus required and suitable light sources. Discuss its application in histopathology.
4. Discuss the histological methods which may be applied to needle biopsies of kidney.
5. Outline the methods for the identification of lipids in tissue sections.

PAPER II

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of seven questions.
3. ALL questions are to be attempted.
4. Mark distribution is shown for each question.

1. Name a normal site and a selective method for the demonstration of each of the following:
(a) Nissl substance; (b) Melanoblasts; (c) Paneth cells; (d) Motor-end plates; (e) Mast cells; (f) Fibrous astrocytes; (g) Bilirubin; (h) Beta cells of pituitary; (i) Beta cells of pancreas; (j) Enterochromaffin cells. (20 marks)
2. State a use for each of the following in a histopathology laboratory:
(a) Alpha naphthylphosphate; (b) Sodium dithionite; (c) Dibutyl phthlate; (d) Ammonium sulphide; (e) Resorcinol; (f) Hydroquinone; (g) Chromic acid; (h) Pyridine; (i) Propylene oxide; (j) Sodium thiosulphate. (10 marks)
3. What substance or staining technique do you associate with the following names:
(a) Best; (b) Schultz; (c) Perls; (d) Loyez; (e) Weigert; (f) Gomori; (g) Sheridan; (h) Lendrum; (i) Bodian; (j) Holzer. (10 marks)
4. State in a few words the basic principle of each of the following techniques
(a) Marchi's technique; (b) Autoradiography; (c) Staining of lipid with Sudan dyes; (d) Turnbull's reaction; (e) Freeze substitution. (15 marks)
5. Define briefly:
(a) Autolysis; (b) Post-chroming; (c) Perfusion fixation; (d) Ice crystal artefact; (e) Double-embedding; (f) Micro-incineration; (g) Chelating agent; (h) Half life of isotope; (i) A leuco dye; (j) A natural dye.
6. Write brief notes on the following:
(a) Oligodendroglia; (b) Argyrophilic plaques; (c) Schwann cells; (d) Purkinje cells; (e) Myelin. (15 marks)
7. What histopathological conditions are associated with the following:
(a) Asbestos; (b) Urate crystals; (c) Silica; (d) Argentaffin; (e) Haemozoin. (10 marks)

PRACTICAL EXAMINATION
HISTOLOGY PART III

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper. At the end of this time, fifteen minutes extra is allowed for writing up only. NO technical work may be done in this time.
2. The paper consists of four questions.
3. All questions are to be attempted.
4. Mark distribution is shown for each question.

1. Cut frozen sections from tissue A and demonstrate alkaline phosphatase by the diazo method. (40 marks)
2. Stain section B by the Solochrome Cyanine method to demonstrate myelin. (10 marks)
3. Serial sections C contain two pigments. What is the tissue and what are the pigments? (40 marks)
4. Prepare specimen D for final museum mounting in the jar provided. (10 marks)

THEORY EXAMINATION
IMMUNOHAEMATOLOGY PART III
PAPER I

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of two sections each containing four questions.
3. Attempt any *TWO* questions from Section A and any *TWO* questions from Section B, plus any *ONE* question from either section (i.e. *five* questions to be attempted).
4. All questions carry equal marks.

SECTION A:

1. "Recent data indicates that blood group glycoproteins may be necessary for the integrity of leucocyte and red cell membranes." Discuss this statement and give specific examples with your answer.
2. Give an account of the linkage or association of blood group genetic markers with disease states and other inherited phenomena.
3. Discuss the enzymatic basis for blood groups in man, using ABH and Lewis blood group systems to exemplify your answer.
4. (a) Discuss transfusion reactions caused by factors other than red cell incompatibility; (b) Describe briefly how the laboratory may be able to assist in avoiding such problems.

SECTION B:

1. In detail, compare the advantages of detecting and quantitating red cell antibodies by automated methods as compared with manual procedures.
2. (a) Outline a scheme for the complete serological investigation of blood from a suspected case of auto-immune haemolytic anaemia; (b) List the antibodies that are known to have been implicated and describe their characteristics briefly.
3. Write notes for a 15 minute lecture to fifth year medical students on the subject of HLA typing and genetics with an explanation of their significance in transplantation.
4. Several antigen/antibody systems have been characterised in viral hepatitis type B (HBV). (a) What are they?; (b) At what stage of disease are they implicated?; (c) What methods are available to detect these systems?

PAPER II

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of 12 questions.
3. ALL questions to be attempted.
4. All questions carry equal marks.

1. Discuss briefly mechanisms of the alternate pathway of complement.
2. Write brief notes on the following: (a) Salis antibody: (b) Rg^a: (c) LW; (d) Fy^x; (e) K_o.
3. What are the functional aspects of Immunoglobulin A (IgA)?
4. Write brief notes on the following substances: (a) Adenine; (b) 2-mercaptoethanol (c) L-cysteine hydrochloride; (d) Sodium glycinate; (e) 10% calcium chloride.
5. What contributions have seed and plant extracts made to immunohaematology?
6. Define or explain the following terms: (a) Chimerism; (b) Syntenic loci; (c) Double back cross; (d) Linkage dis-equilibrium; (e) Crossing over.
7. Describe briefly a screening test for syphilis. Comment on the causes of false positive reactions.
8. What is polymorphism? Using specific examples, outline briefly three methods that will demonstrate this phenomenon.
9. Write brief notes that will help to distinguish between the following pairs: (a) V and VS; (b) r^Mr and r^Gr; (c) Anti Rhi and Anti CE; (d) Rh_{null} and Rh_{mod}.
10. Choose a blood group system in which you know the percentage frequency of two antithetical antigens and, showing your working, calculate the expected gene frequency of each allele.
11. What advantages does Acid citrate dextrose (ACD) have over both Tri-sodium citrate and Heparin in the storage of blood for transfusion?
12. Write brief notes on the relative value of direct and indirect methods for platelet antibody detection.

PRACTICAL EXAMINATION
IMMUNOHAEMATOLOGY PART III

INSTRUCTIONS:

1. Time allowed: Three hours for practical work. Ten minutes extra is allowed for reading this paper and familiarisation with equipment. At the end of this time, fifteen minutes extra is allowed for writing up only. NO technical work may be done in this time.
2. *BOTH* questions to be attempted.
3. Mark distribution is shown for each question.

1. Using the extract Ulex europaeus seeds provided, classify the saliva samples as secretors or non-secretors of H substance. (25 marks)
2. You are supplied with 2 sets of maternal/infant clotted blood samples. *In each case*: (a) The baby has developed jaundice, and; (b) The mother has not had any antenatal care. Perform relevant serological investigation which will disclose the possible cause of the elevated bilirubin levels in infants. Comment on your findings. (75 marks)

THEORY EXAMINATION
IMMUNOLOGY PART III
PAPER I

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of eight questions.
3. Answer *FIVE* questions only.
4. All questions carry equal marks.

1. Explain the theory and practical applications of antigen/antibody reactions.
2. You are presented with a serum and urine from a patient suspected of having myelomatosis. Describe the tests you would undertake to confirm or exclude that diagnosis.
3. Discuss the interaction of antibody, complement and the cellular components of the immune system in host defence mechanisms directed against pyogenic bacteria and viruses.
4. A variety of assays for antibodies to Brucella and Leptospira antigens are available. Describe these and discuss their interpretation.
5. The characterisation of the immunoglobulin molecule has been one of the major achievements of contemporary immunology. Discuss the characteristics and biological activities of these proteins.
6. Antibodies against streptococcal antigens are valuable in the diagnosis of rheumatic fever. Discuss the technique for detection of these antibodies and give your interpretation of the results.
7. Discuss the circumstances where the host is damaged by its own immune system.
8. You are presented with a patient suspected of having a deficiency of the complement system. Describe appropriate analyses you would carry out to investigate this patient and the interpretation of the results of the analyses. Tests outside the scope of your own laboratory but appropriate to the investigation may be discussed.

PAPER II

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. *ALL* questions to be answered. There are forty short questions listed under six main question headings.
3. All short questions carry equal marks. Marks for each of the six questions are shown.

1. Interpret and comment on the following groups of results. Each group is the results of a single patient.
   (a) VDRL, 1:2; WR, Positive; FTA abs., Positive. (b) VDRL, 1:4; WR, Positive; FTA abs., Negative. (c) ANF, 1:128 (speckled); CH_{50}, Depressed; C3, 0.4 g/l (normal range 0.8-1.4 g/l); C4, 0.1 g/l (normal range 0.2-0.5 g/l). (d) Smooth muscle antibodies, Positive 1:20. (e) T lymphocytes (SRBC rosettes), 10%; B lymphocytes (Surface Ig), 80%; Peripheral blood white cell count, 12.0×10^9/l 70% lymphocyte. (12½ marks)
2. Write short notes on:
   (a) Null cells; (b) Secretory IgA; (c) Yaws; (d) F_c receptor; (e) Bence-Jones protein; (f) C-reactive protein; (g) IgM antitoxoplasma antibodies; (h) Phytohaemagglutinin; (i) C1 esterase inhibitor; (j) IgE. (25 marks)
3. Briefly indicate the principles of the following assays:
   (a) Nitroblue tetrazolium Test with neutrophils (NBT); (b) Radioallergosorbent Test (RAST); (c) Farr technique; (d) Radioimmuno-assay; (e) Mixed lymphocyte reaction. (12½ marks)
4. Discuss briefly:
   (a) Serum sickness; (b) Cobra venom factor; (c) Alternate complement pathway; (d) Lymphokines; (e) Immunological tolerance; (f) Rheumatoid factor; (g) Transfer factor; (h) Distinction between avidity and affinity of an antibody; (i) Atopy; (j) Australia antigen. (25 marks)
5. The following questions are to be answered with a list only and no further comments are required.
   (a) Conditions in which C3 nephritic factor occur?; (b) Laboratory tests for cell-mediated immunity; (c) Four endocrine diseases associated with auto-antibodies; (d) Further investigations required for a patient who presents with marginal infectious mononucleosis titres; (e) Tests of help in the diagnosis of rheumatic fever. (12½ marks)
6. Supply the following information:
   (a) Draw a diagram of a lymph node (labelled); (b) What are HLA and H-2 antigens?; (c) What is the derivation of the name VDRL?; (d) Distinguish between Fab and $F(ab)_2$ fragments; (e) Allogeneic transplants represent what relationship between donor and recipient? (12½ marks)

PRACTICAL EXAMINATION
IMMUNOLOGY PART III

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper. At the end of this time, fifteen minutes extra is allowed for writing up only. NO technical work may be done in this time.
2. The paper consists of four questions.
3. All questions are to be attempted.
4. All questions carry equal marks.

1. Determine the titre of anti-nuclear antibody in the serum sample provided. The serum is from a patient with SLE.
   Comment on any unusual features. See method sheet 1.
2. Determine the titre of antibody in the serum sample provided (anti-bovine-serum-albumin) by passive haemagglutination using bovine serum albumin cupled to tannic acid treated erythrocytes. For details see method sheet 2.
3. In this exercise you are required to determine a protein selectivity index using two proteins, human IgG (M.W. 160,000) and transferrin (siderophilin M.W. 80,000). The derivation of the protein selectivity index is given in method sheet 3.
   Serum and urine samples from a patient with proteinuria have been analysed using a partigen plate.
   In selective proteinuria the urinary proteins consist essentially of albumin and possibly a small proportion of low molecular weight plasma globulins. In non-selective proteinuria there is

a variable amount of most of the plasma globulins including the macroglobulins. See method sheet 3.

4. (a) Estimate C3 levels from the radial immunodiffusion plate provided.
   Wells 1-5 have been filled with serum samples (diluted 1:2) from patients 1-5 respectively whose clinical and laboratory details are given in the following section.
   Wells, 6, 7 and 8 have been filled with C3 standard preparations containing 33mg, 65mg, 127mg1/0ml (normal 80-140mg/100ml).
   (b) Clinical and laboratory details relating to the 5 individual patients whose C3 levels you have just estimated are given below.
   Give your interpretation of the results and your suggestions for further investigations.
   Patient 1: Butterfly rash, anti-nuclear antibody 1:512 with homogeneous staining pattern, complement not detected by haemolytic assay, C4 markedly decreased, LE cell test negative.
   C3 result =
   Patient 2: Episodic urticaria haemolytic complement decreased, C4 marked decrease.
   C3 result =
   Patient 3: SLE on treatment, anti-nuclear antibody 1:64, homogeneous peripheral staining complement not detected, C4 low normal, DNA binding increased.
   C3 result =
   Patient 4:? Membranoproliferative glomerulonephritis C4 normal, haemolytic complement reduced.
   C3 result =
   Patient 5: Lepromatous leprosy with erythema nodosum during treatment, haemolytic complement not detected, C4 normal.
   C3 result =

THEORY EXAMINATION
MICROBIOLOGY PART III
PAPER I

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of six questions.
3. Questions 1, 2, 3, 4 and *either 5 or 6* are to be attempted.
4. All questions carry equal marks.

1. Give an account of the factors you consider of importance in ensuring laboratory safety and staff protection. Include comments on:
   (a) Physical protection; (b) Use of disinfectants; (c) Disposal of infected material; (d) Aerosols; (e) Restriction of dangerous pathogens; (f) Staff health and accidents.
2. Write a *brief* account on *four* of:
   (a) Methods for detecting Hepatitis B Surface antigen (HB_sAg); (b) An "in-use test" of disinfectants; (c) Common causes for the failure of disinfectants as used in hospitals; (d) The specimens required and the methods of examination to diagnose Schistosomiasis; (e) The specimens required and the initial cultural methods used for the isolation of *Mycoplasma pneumoniae.*
3. Write short notes on *five* of the following:
   (a) Rota-virus (b) *Haemophilus influenzae* serotypes; (c) Amikacin sulphate; (d) 5 Fluorocytosine; (e) Chlamydia; (f) *Giardia lamblia* pathogenicity; (g) *Streptococcus agalactiae.*
4. What antibiotic sensitivity testing method would you recommend for routine use in a hopsital laboratory? Discuss the reasons for your choice under the following headings:
   (a) Media and methodology; (b) Materials and cost; (c) Controls; (d) Recording and intepretation of results.
   Against which anti-microbial agents, if any, would you test the following:
   (a) Pseudomonas from sputum; (b) *Haemophilus influenzae* from cerebrospinal fluid?
5. Discuss the dermatophytes of human importance classifying the species into the groups:
   (a) geophilic; (b) zoophilic; (c) anthropophilic. Name two species in each group and give details of how you would identify three species selecting one from each group.
6. Discuss the medically important yeasts, the infections they may cause and the methods you would use for their recognition, isolation and identification.

PAPER II

INSTRUCTIONS:

1. Time allowed: Three hours. Ten minutes extra is allowed for reading this paper.
2. The paper consists of four questions in each of which there is a choice.
3. *One* of the alternatives in *all four* questions is to be attempted.
4. All questions carry equal marks.

1. (a) (i) Write an account of the morphology and life cycle of *Toxoplasma gondii.* (ii) Discuss its transmission in the community; (iii) Discuss the laboratory tests available and their interpretation for the diagnosis of toxoplasma infection.

OR (b) (i) Discuss the epidemiology of Amoebic Meningoencephalitis; (ii) Describe the morphology of the causative organism; (iii) Discuss the laboratory procedures you would use to make the diagnosis and the expected findings in a typical case.

2. (a) (i) Discuss the current role of "Opportunistic" pathogens in the hospital environment; (ii) What are some of the types found in many hospitals and what part may antibiotic policy play in their control?

OR (b) (i) Discuss the infection hazards in the administration of various types of parenteral solution; (ii) Describe in detail the investigations you would make to establish the cause of a number of patients developing bacteraemia following administration of parenteral solutions.

3. (a) Filamentous gram positive and gram negative bacilli are seen in the direct smear of foul smelling pus from a brain abscess.
   Describe in detail the procedures you would use to isolate and identify the possible causative organisms.

OR (b) (i) Discuss the reasons why some *Escherichia coli* strains may cause diarrhoea; (ii) What laboratory procedures may be used to recognise and identify these enteropathogenic strains? (iii) What

significance would you attach to the isolation of these *enteropathogenic E. coli* from an infant and an adult?

4. (a) (i) Discuss the several techniques which may be used for the demonstration of antigen or antibody by precipitation in gel; (ii) What are their advantages, disadvantages and applications?; (iii) Describe in detail how one such test is performed and how it is interpreted.

OR (b) (i) Discuss the prinicples of the different types of haemagglutination tests; (ii) List some of their applications and give reasons for their widespread use; (iii) Describe in detail how one of these tests is performed and its interpretation.

PRACTICAL EXAMINATION MICROBIOLOGY PART III

INSTRUCTIONS:

1. Read the Cover Sheet and paper CAREFULLY.
2. Time allowed: Three hours—first day; two hours—second day.
3. The paper consists of six questions.
4. Answer all questions.
5. Mark distribution is shown for each question.

COVER SHEET

Answer sheets are provided which must be completed before the end of each day's practical. Sufficient space is available to answer questions. Extra paper should not be necessary. The answer sheets completed on the first day will be collected and *not returned.*

Further answer sheets for the final results will be provided on the second day.

Rough notes and working notes for your own use should be written in the book provided. *This will not be assessed by the examiners.*

1. Disinfectant is provided in the glass containers for disposal of infected material. If a culture is split on the bench, inform a supervisor.
2. *Microscopes*: Each candidate is provided with a binocular "Olympus" microscope of identical design.
3. *Gram Stain*: Decolourising agent is acetone/alcohol.
4. An initial supply of some reagents and media are on your bench. These will not be sufficient and additional reagents or cultures will be provided on request. Raise your hand to attract supervisor's attention. Do not leave your place.
5. All smears and other preparations should be labelled and left for inspection by the examiners.
6. Cultures and titres for incubation should be clearly marked with the candidate's number, culture identification and the atmosphere and temperature for incubation.
7. Please remember to bring this examination paper with you to the practical examination tomorrow.

IMPORTANT: Please note: These questions and the relevant part of the answer sheet are to be read in conjunction. The questions below give you information about the specimens and the extent of the examination required.

The answer sheet provides space to record your findings and may ask additional questions.

1. The sera labelled 1A and 1B are to be tested for the presence and level of *Brucella abortus* antibodies by:
(a) Standard Agglutination Technique *and;* (b) Complement Fixation Technique.
The necessary information about reagent concentrations is in the instructions on the answer sheet.
(24 marks)

2. These organisms are to be identified as far as practicable:

A. Culture from a faeces of four year old child with diarrhoea.
(API strip, sterile water, sterile pasteur pipettes, motility medium are provided.)

B Culture from a sputum of a 75 year old male with pneumonia.
(API strip, sterile water, sterile pasteur pipettes, motility medium are provided.)

C. Culture on chocolate agar isolated from a throat swab.
(Please note on answer paper any additional materials that you may require. The supervisors will provide available material for sub-cultures.)

D. A blood agar plate that has been incubated aerobically for 48 hours. The specimen inoculated was from a vaginal discharge of a female 36 weeks pregnant.
(The supervisors will provide additional materials for identification/sub-culture on request.)

E. This is a sub-culture of an organism that was recovered from a blood culture after 10 days incubation. The plate has been incubated at 37°C in 10% CO_2.
What is your provisional identification and what additional tests would you set up to confirm your initial identification?
(*Note*: NO sub-cultures are necessary with this specimen.) (25 marks)

3. All plates have been incubated anaerobically for 24 hours.
Organisms:

3A Isolated in pure culture from necrotic tissue on the lower leg of a male following an accident with a motor mower.

3B Isolated from a post-appendectomy wound abcess.

3C Isolated from a rapidly developed necrotic lesion of the index finger.

3D Isolated from an abcess in the neck.
Examine these cultures and:
(a) Indicate your provisional identification of each organism; (b) What further tests would you perform, and what results would you expect, to allow a rapid confirmation of your initial identification?; (c) What initial reports would you issue that could be useful to the clinician prior to final confirmation?
Note: You are *NOT* required to undertake any further sub-cultures of these organisms.
(16 marks)

4. These two API strips, motility media and blood agar plates labelled 4A and 4B are of organisms that have been isolated from the faeces of a six month old baby with diarrhoea and vomiting.
Record the results from the API strips on the the answer sheet. Do further tests, *not involving sub-cultures,* to complete the identification.
(10 marks)

5. Organisms labelled 5(A), 5(B), 5 (C) and 5(D) are to have sensitivity tests carried out against the supplied discs using standard procedures. The discs to be used are:
   Ampicillin; Cephalothin; Gentamicin; Sulphamethoxazole.
   Broths are provided for inoculum, sterile cotton-tipped applicators are provided for inoculation of the plate medium.
   Please record on answer sheet details of your methods of preparing the inoculum and inoculating the medium. (15 marks)
6. Materials for this question will be provided tomorrow. The question itself will be given in the instructions on tomorrow's answer sheet.
   (Examine the faecal concentrations 6A, 6B and 6C and report your findings on the answer sheet.)
   (10 marks)

The Library

List of Current Periodicals

Librarian: Miss M. Johnstone, Microbiology, Dunedin Public Hospital.

Amer. J. med. Technol.
Vol. 42, No. 12, December, 1976.
Vol. 43, No. 1, January, 1977.
Contents: Septicaemia Due to *Campylobacter fetus* in a Newborn Infant with Gastroenteritis; Cryotomy Techniques.
Vol. 43, No. 2, February, 1977.
Contents: Determination of Inorganic Phosphorus Using a Centrifugal Analyser; A Review of Serologic Problems Caused by Polyagglutinable Red Cells: Clinical Parasitology 1975.

Aust. J. med. Technol.
Vol. 8, No. 1, February, 1977.
Contents: Methods and Techniques for Reducing Failure Through Rejection by the Recipient in Organ Transplant Surgery: Meningitis and Septicaemia Caused by *Pasteurella multocida;* An Uncommon Case of Trichostrongylosis in the Illawarra area of NSW.

Jena Review
No. 6, 1976.
No. 1, 1977.

J. Amer. med. Technol.
Vol. 39, No. 1, January-February, 1977.
Contents: A Chromogenic Characteristic of an Aerobic Pseudomonad Species in 2% Tryptone (Indole) Broth; Novel Quality Assurance Procedure for the Gram Stain; A Simple Method of Preserving Bacteria.

Lab. Med.
Vol. 8, No. 1, January, 1977.
Contents: Pathophysiology of Insulin Secretion; Glucagon and its Clinical Significance; Cytomorphologic Changes in the Leukocytes of Patients with Malignant Disease.
Vol. 8, No. 2, February, 1977.

Medical Biology
Vol. 54, No. 5, December, 1976.

Problems in Haematology and Blood Transfusion
(All articles in Russian.)
Nos 1-3, 1977.

Sth Afr. J. med. Lab. Technol.
Vol. 22, No. 3, September, 1976.
Contents: The Cryostat-Application of Sections Produced; Comparison of the Abbott Competitive Protein Binding and Radioimmunoassay in the Determination of Serum Thyroxine: *Klebsiella ozaenae* Septicaemia.
Vol. 22, No. 4, December, 1976.
Contents: Work-Simplified Determination of Urinary Creatinine; Kinetic Determination of Serum Alkaline Phosphatase Using Phenolphthalein Monophosphate as Substrate.

Amer. J. med. Technol.
Vol. 43, No. 3, March, 1977.
Contents: An Improved Turbidimetric Method for Plasma Fibrinogen; Pathogenesis and Epidemiology of Opportunistic Mycotic Infections: A Review; Autoimmune Diseases; Non-parametric Percentile Estimate of Clinical Normal Ranges.

Canad. J. med. Technol.
Vol. 39, No. 2, April, 1977.
Contents: An Investigation of Staining Techniques for Use in Automated Cytology Screening; Comparative Evaluation of Reagents for the Activated Partial Thromboplastin Time in the Assay of Coagulation Factor VIII and IX; Cytologic Diagnosis of Thyroid Lesions by Fine Needle Aspiration Biopsy; Identification of Group A Streptococci by a Fluorescent-Antibody Technique and by a Bacitracin Sensitivity Method; Human Chorionic Gonadotrophin and its Detection by Immunochemical Methods.

The Gazette
Vol. 21, No. 3, March, 1977.

Jena Review
No. 2, 1977.

Lab. Delo.
No. 2, 1977.

Lab. Med.
Vol. 8, No. 3, March, 1977.

Medical Biology
Vol. 54, No. 6, December, 1976.

Revista del. Viernes med.
Vol. 27, No. 2, May-August, 1976.

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Microbiology—1976, Schlessinger ... 50

Directions for Contributors

These instructions are provided with the object of ensuring uniformity of presentation. Manuscripts should be typed double spaced, on one side only of good quality paper with one inch margins. Carbon copies are not acceptable. Give the author's name with initials if male, or one christian name if female, and the address of the laboratory where the work was carried out. Use capitals only where indicated and do not underline except where italics are required.

In general, papers other than reviews, should consist of a short summary capable of standing alone as an abstract; an Introduction (outlining the problem and the proposed solution); Material and Methods; Results and Discussion.

Illustrations

Illustrations are costly and should be used sparingly. Graphs, line drawings and photographs are all referred to as 'Figures' and should be numbered in the order of their appearance in the text using arabic numerals. Drawings (in indian ink on stout white paper) and photographs, should be about twice the size of the actual reproduction. The position of figures in relation to the text should be noted in the typescript. Legends typed on separate sheets are numbered to correspond with the illustrations. Tables should be typed separately and numbered in roman numerals.

Nomenclature and Units

Scientific names of micro-organisms should conform with Bergey's Manual of Determinative Bacteriology. The first time an organism is mentioned the full generic name should be given and underlined to indicate that it is to be printed in italics. Subsequently it may be abbreviated. Trivial or common names are printed in roman, e.g., staphylococci, and should not be underlined.

To conform with the Systemes Internationale D'Unites or SI units it is recommended that the following prefixes and abbreviations be employed.

Length: m, cm, mm, μm, nm.
Area: m^2, cm^2, mm^2, μm^2.
Volume: litre, ml, μl, nl, pl ('litre' in full avoids confusion with 'l')
Mass: kg, g, mg, μg, ng, pg.
Mass concentrations: kg/litre, g/litre, mg/litre, μg/litre. For the present concentrations per 100 ml also accepted as are daily outputs in urine and faeces.
Molar concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre. (For the present mequiv/litre may also be used.)
Temperature: Express as °C.
Time: s, min, h, d, a. The latter two symbols which stand for day and year respectively are best expressed in full to avoid confusion.
Density: kg/litre (relative density replaces 'specific gravity')
Clearance: litre/s, ml/s (for the present ml/min may also be used).

N.B.:

1. The symbol for a unit is unaltered in the plural and should not be followed by a full stop, e.g., 5 cm not 5 cm. nor 5 cms.
2. No space should be left between the symbol for a prefix and the unit. A space is left between the symbols in derived units,
   e.g., ms = millisecond
   m s = metre x second
   Where ambiguity could arise abbreviations should be written in full.
3. **Numbers.** The decimal is indicated by a full stop. Commas are not used to divide large numbers but a space is left after every third digit.

A zero should precede numbers less than unity. Units which give a number between 0.1 and 1000 should be chosen when possible.

References

References should be listed alphabetically at the end of the article and numbered to correspond with the numbers used in superscript within the text. Citations in the text should give the author's name using *et al.* if more than one author, and the year, thus: Walker *et al.* (1972)[1]. All authors' names should be listed with initials; year of publication in brackets; journal title abbreviated and underlined to indicate italics; volume number in arabic numerals underlined with a wavy line to indicate bold type and the first page number. The reference for abbreviations is the World List of Scientific Periodicals. In general nouns have capitals, adjectives do not and conjunctions are omitted. Authors are referred to previous journals for examples.

Proofs

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