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# ANNOUNCEMENT

LAB NEWSLETTER No. 1 MARCH, 1977

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# A Case of Thrombotic Thrombocytopenic Purpura

**B. W. Main**

**Haematology Department, Dunedin Hospital**

*Based on a paper read at the NZIMLT Conference, Whangarei, 1976*

## Introduction

Thrombotic thrombocytopenic purpura is a rare disorder of unknown aetiology characterised by the occurrence of fever, thrombocytopenic purpura, haemolytic anaemia, fluctuating neurological disturbances and renal disease. It is of rapid onset and abdominal pain and jaundice are often present. It may occur at any age but is most common in young adults. Blood examination shows a severe haemolytic anaemia, thrombocytopenia and leucocytosis, with the typical morphological feature of microangiopathic haemolytic anaemia present. It is almost invariably fatal.

## Case History

The case described had all of these features.

The patient, a female, aged 28, was admitted on 8th June from a peripheral hospital with a four-day history of an influenza-like illness, diarrhoea for three days, and cyanosis and bruising for 24 hours. On examination she was found to have been anuric for 24 hours, had extensive bruising and cyanosis and was complaining of widespread pain particularly abdominal pain.

The cyanosis was seen to be affecting the fingers and lower limbs but these were not centrally cyanosed. There were petechial haemorrhages over the arms, legs, front of trunk, face and particularly the cheeks where there was confluent petechial haemorrhage. The patient was tender in both renal angles and there was a necrotic lesion in the centre (L) calf. No neurological deficiencies were noted at this time.

Laboratory investigations showed a microangiopathic haemolytic anaemia with marked thrombocytopenia (Fig. 4) and high urea, bilirubin and glucose levels. The haemoglobin of 119 g/l fell rapidly to 76 g/l over 3 days and the blood urea which was 6.4 mmol/l on the day before admission climbed to 49.7 mmol/l in the subsequent 3 days. Platelets were 12 x $10^9$/l and gradually improved after the commencement of therapy. Coagulation

**Figure 1.** — Cyanosis of fingers.

**Figure 2.** — Confluent petechial haemorrhage of the cheeks.

**Figure 3.** — Petechial haemorrhage on the legs with cyanosis of feet.

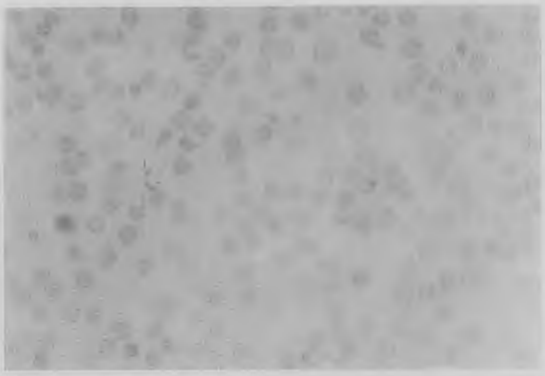

Figure 4. — Microangiopathic haemolytic changes in the red blood cells.

results showed increased fibrin degradation products, but the fibrinogen was normal. Prothrombin and thrombin clotting times were only slightly elevated and returned to normal following therapy.

*Therapy*: The patient was given heparin 4000U I.V. four hourly with hydrocortisone and insulin for the high glucose. Two days after admission the patient was placed on a respirator because of respiratory distress. Three days after admission renal dialysis was commenced following the insertion of scribner shunt. Blood transfusion was given for anaemia and heparin increased to 6000U. At this stage no further skin purpura had occurred and cyanosis was improving. Six days after admission further improvement in purpura and cyanosis was noted. Nine days after admission the patient was still anuric and tracheostomy was performed for permanent attachment to a respirator and she was commenced on Di pyridamole (a platelet inhibitor). Problems were encountered however in controlling the heparin therapy and oozing from the tracheostomy wound so the Di pyridamole was discontinued. The following day purulent sputum was produced indicating a respiratory infection and klebsiella was isolated.

The patient's general condition continued to worsen and she died on 21/6, 13 days after admission.

Postmortem examination showed extensive haemorrhage and necrosis in almost every organ with infarcts in the brain, heart and lungs. The kidneys showed extensive cortical necrosis with fibrin thrombi in the venules and glomerular capillaries.

**Summary**

This patient showed the classical symptoms of thrombotic thrombocytopenic purpura which in spite of intensive supportive therapy and considerable improvement of the purpura and peripheral cyanosis, culminated in death due to irreversible damage to the kidneys and other organs and subsequent infection.

**Acknowledgment**

I am indebted to Dr L. A. Bates, Clinical Haematologist, for permission to publish this case history.

# The Sensitivity of *C. albicans* to 5-Fluorocytosine

**Sharon Vaughan**

**Microbiology Department, Green Lane Hospital, Auckland**

*Based on a paper read at the NZIMLT Conference, Whangarei, 1976*

**Introduction**

Due to the increased use of 5-fluorocytosine it was decided to determine the degree of resistance of *Candida albicans* species isolated at Green Lane Hospital to see how our results compared with other literature available. Steer *et al* (1972)[4] in their paper in Annals of Internal Medicine found that 92% of *C. albicans* strains were inhibted by 1.95 μg/ml 5FC, whereas an article by the American Society of Hospital Pharmacists (1974)[1] entitled Antifungal Antibiotics, showed 20-25% of *C. albicans* isolated had a minimum inhibitory concentration of 5FC greater than 100 μg/ml.

5FC is a fungistatic drug that acts as an anti-metabolite by competing with cytosine and related pyrimidines in fungal metabolism.

The main indications for the use of 5FC are acute systemic and cutaneous candidosis, cryptococcosis, chromoblastomycosis, and conditions caused by other sensitive fungi.

It is well absorbed orally and good levels may be obtained in the tissue, blood, cerebro-

spinal fluid and urine. A dosage for a 70kg adult of 50 mg/kg every six hours results in a trough and peak of 30 and 75 μg/ml respectively[2]. 250 mg/kg/day for 3 weeks has been shown to produce no toxicity[3]. In vitro studies have shown that an increase in resistance tends to occur if serum levels fall below 25 μg/ml[2].

## Materials and Methods

The medium used to test 5FC sensitivity must be completely synthetic without animal protein substances as these have been shown to inhibit 5FC activity 20 fold, e.g. in standard complex animal protein mycological media such as Sabdex agar[4].

Cultures of *C. albicans* that were isolated at Green Lane Hospital from clinical specimens during routine culture were collected on Sabouraud's Dextrose Agar slopes. Each isolate was identified by germ-tube production in serum after 3 hours at 37°C, and then confirmed by chlamydospore production on Fragners agar after 48 hours at 27°C.

Each fresh culture was inoculated by a straight wire into 2.5 ml of saline to give a concentration of approximately 1 x $10^3$ yeast cells/cmm. A sterile swab was then used to inoculate the yeast suspension in a line across a yeast morphology agar plate. Five test suspensions and one control *C. albicans* of known M.I.C. were inoculated in this way in parallel lines, on one plate.

A 5FC solution of 100 μg/ml was made in yeast nitrogen base supplemented with L-asparagine and glucose. A sterile 70 x 6mm strip of Whatman Number One filter paper was soaked in the solution and allowed to dry. This strip was placed down the centre of the plate at right angles to the yeast inocula, and the plates were incubated at 37°C for 48 hours. Zone sizes that compared with the control were read as sensitive and any yeast cultures that gave doubtful zones of inhibition were rechecked for sensitivity to 5FC by the tube dilution technique. The yeast to be tested was inoculated into yeast nitrogen base and incubated at 37°C for 3-4 hours. It was then diluted to a concentration of 1x$10^3$ yeast cells/cmm with further yeast nitrogen base (calculated using a Neubauer Counting Chamber).

The 5FC was diluted in yeast nitrogen base to give a concentration range of 100 μg/ml down to 0.05 μg/ml. A drop of the diluted yeast suspension was added to each tube including a growth control tube and the test was incubated 48 hours at 37°C.

## Results

Out of 170 isolates of *C. albicans* tested, only one was found to be resistant to 100 μg/ml 5FC. This yeast was isolated from a scrotal swab taken from an elderly man who was known to have had no previous exposure to 5FC during his stay at G.L.H. A further 6 isolates showed doubtful sensitivity using the strip diffusion technique, i.e. they grew completely up to or close to the strip. These isolates, when checked using the tube dilution technique, gave a range of sensitivity to 5FC of 0.4 to 1.6 μg/ml.

## Conclusion

From the results obtained at Green Lane Hospital we found that more than 99% of the *C. albicans* isolated were sensitive to 5FC. It would appear then that due to its ease of administration, levels achievable and low toxicity, 5FC may be the drug of choice for treatment of systemic candidosis.

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# The Preparation of Leucocyte Homogenates for Enzymatic Assays

**Jenny G. Perrow**

**Biochemistry, Green Lane Hospital, Auckland.**

*Submitted as a thesis in partial fulfilment of the examination for Qualified Technical Officer, April 1976*

## Summary

The preparation of homogenates of human leucocytes is described. The homogenates produced contain the constituents from one to two million leucocytes in a final volume of 0.4 to 0.5 millilitres. The method involves the collection of micro-samples (0.1-0.2 ml), in heparinised capillary tubes, and centrifugation to obtain the buffy layer. This leucocyte-rich preparation is further purified by density gradient centrifugation. The isolated leucocytes are then homogenised in an all glass micro-homogeniser. (Duall).

## Introduction

In the living cell, glycogen synthesis and degradation proceed by separate pathways[8], each pathway being biologically important (as shown in Figure 1). There is a complex inter-relationship between the activity of the enzyme 'glycogen synthetase', which controls synthesis, and the activity of phosphorylase, which determines the rate of glycogen breakdown. The first step in glycogen synthesis is the reversible conversion of glucose-1-phosphate to uridine diphosphoglucose (UDPG), by a specific pyrophosphorylase (as shown in Figure 1). The inactive form of glycogen synthetase becomes active in the presence of glucose-6-phosphate[8]. In the complete degradation of glycogen, at least 2 enzymes, phosphorylase and 'debranching enzyme', amylo-1, 6-glucosidase, have to act in proper sequence. Phosphorylase attacks only the 1, 4 glucosidic linkages of the outer chains of the glycogen molecules, with the formation of glucose-1-phosphate.

Deficiencies of any of these enzymes involved in glycogen metabolism may result in the formation of abnormal glycogen, or the accumulation of normal glycogen. There are six major types of glycogen storage diseases[8], Von Gierke's Disease (Type 1 Glycogenosis) is the commonest form[10]. It is caused by a deficient activity of glucose-6-phosphatase, affecting the liver, kidney and gut, and results in hepatomegaly. The other types affect liver, heart or muscle, and can be treated. However, Pompe's Disease, Type II Glycogenosis, a recessive disorder caused by the deficiency of the lysosomal, $\alpha$-1, 4-glucosidase[2], (acid maltase deficiency), is fatal within the first two years of life.

In this disease there is a progressive accumulation of glycogen inside vacuoles related to the lysosome system, and a relatively normal pattern in the surrounding cytoplasm[6]. Deposition of glycogen in Pompe's disease is generalised, but particularly affects the heart, tongue and brain. The clinical features are cardiomegaly with heart failure, enlarged tongue and muscle weakness. As an aid in diagnosis, disturbances of carbohydrate or lipid metabolism are remarkable by their absence (as shown in Table I).

TABLE I.—Summary of the metabolic responses in the six types of glycogen storage disease[8].

| Type | Change in blood sugar and lactic acid in response to: Fasting | Fructose or Galactose | Glucagon | Glycogen structure | Fat Metabolism | Blood lactate after lactic acid exercise |
|---|---|---|---|---|---|---|
| I | Glucose<br>Lactic acid | -<br>++ | Glucose<br>Lactic acid | 0<br>++ | Normal | All plasma lipids very high | Normal |
| II | Glucose<br>Lactic acid | +<br>- | Glucose<br>Lactic acid | +<br>! | Normal | Normal | Normal |
| III | Glucose | - | Glucose<br>Fasting<br>Post prandial | ?<br>- | Short outer chains | High fasting TG | Normal |
| IV | Glucose | + | Glucose | I | Very long outer chains | Not reported<br>High TG | Not reported |
| V | Glucose<br>Lactic acid | -<br>+ | Glucose<br>Lactic acid | +<br>+ | Normal | Uptake by muscle after exercise. | Normal |
| VI | Glucose | + | Glucose | 0 | Normal | High cholesterol and neutral fats. | Normal |

0 = No increase  I = Poor increase
+ = Normal increase  ++ = Excessive increase

Leucocyte enzyme studies, however, allow diagnosis of this disease in babies. The existing methods of determining $\alpha$-1, 4-glucosidase activity, require 10 ml of blood. Obtaining volumes of this size from small, sick children presents difficulties. Children presenting with large hearts is a reasonably frequent occurrence at Green Lane Hospital. To eliminate the possibility of Pompe's disease as a diagnosis, it is desirable to screen all these children, a large proportion of whom are normal. To

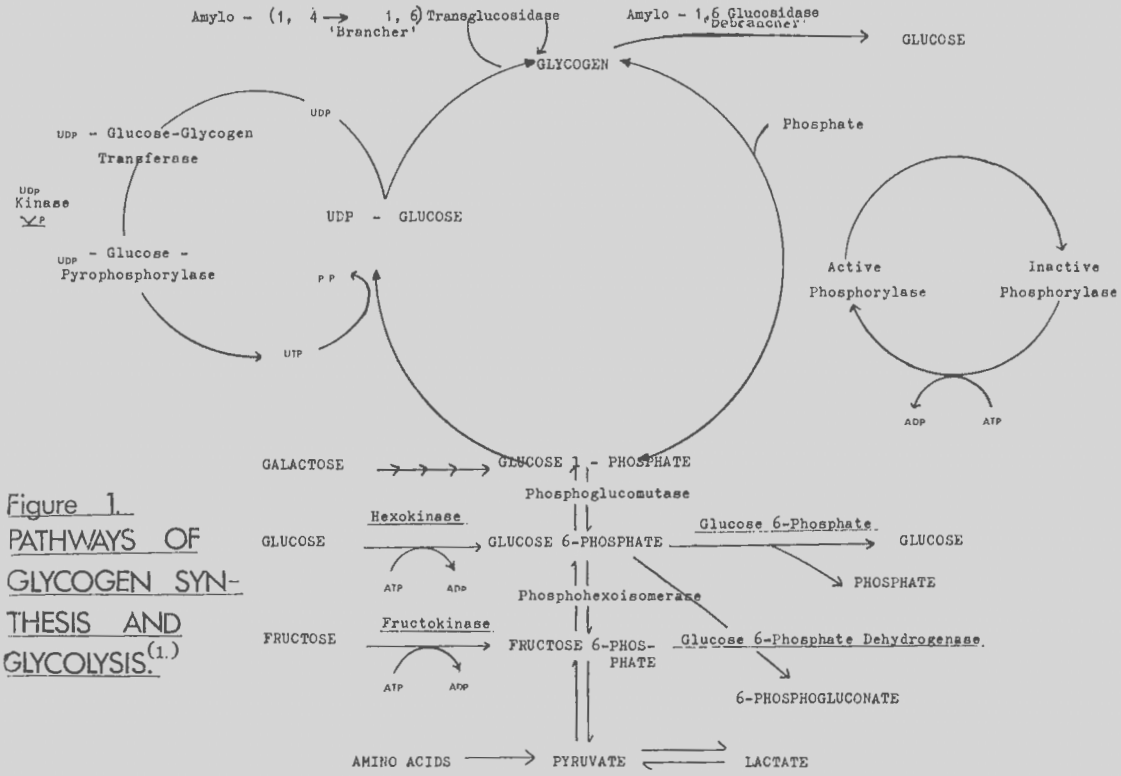


Figure 1. PATHWAYS OF GLYCOGEN SYNTHESIS AND GLYCOLYSIS.[1.]

simplify sample collection, a micro method for determining the leucocyte $\alpha$1, 4-glucosidase would be of considerable assistance. The first step is to devise a method for obtaining a suitable micro sample. The purpose of this study is to show how a preparation of leucocytes, free from erythrocytes, can be obtained from a heparinised capillary sample.

Gradient centrifugation, a technique used in this study, involves the separation of the various cellular components from each other, by centrifugation in a supporting medium that is close to isotonic[4], but contains zones of different density. Under centrifugation, particles that differ in density, or in size (or shape), sediment at different rates in an applied field of centrifugal force, i.e., a gravitational field. When a particle reaches a zone of density equal to that of the particle, it will not be sedimented further, i.e., the different types of particles separate into zones characterised by their mean sedimentation coefficients. Rapidly sedimenting fractions collect as a pellet at the bottom of the tube. Lighter fractions will be distributed in zones depending on the density of the supporting medium. This technique is particularly useful for separating different populations of cells[5].

**Procedure:**

A. Reagents:

— *Ficoll Solution.*

1. Dissolve 11.85 grams of Ficoll (Pharmacia) in 105 ml of distilled water.
2. Add 20 ml of Conray 280 (X-ray contrast medium), and mix.

— *Medium 199.*

This was prepared from 'Difco' culture medium 10X strength, and stored at 4°C prior to use.

— *Alkaline Copper Tartrate for Protein Determination.*

Mix immediately prior to use, 25 ml of alkaline carbonate with 0.5 ml of copper sulphate solution.

Alkaline Carbonate — (2% sodium carbonate in 0.1 M sodium hydroxide). Copper Sulphate

Solution — Dissolve one gram of sodium potassium tartrate in distilled water. Add 0.5 grams of copper sulphate, dissolve, and dilute to 100 ml.

— *Folin — Ciocalteau Reagent.*

This is prepared as described by Varley[9], and adjusted to an acid strength of 1.0 Normal.

— Saline, (9 gm/litre).

B. **Methods:**

— *Leucocyte Purification Procedure.*

For this procedure glass micro tubes 14-16 centimetres in length, and 0.2 centimetres internal diameter, were filled from well-mixed heparinised blood. Each tube holds about 100 microlitres of blood. These were centrifuged at 3000 r.p.m. for 10 minutes. The buffy layer, together with a small number of red cells, and a little plasma, was then obtained from two micro tubes, by cutting the tubes above and below this layer. This layer was mixed with 0.2 ml of Medium 199 in a small, plastic autoanalyser cup (1.8 ml size). To a one centimetre by seven centimetre glass tube was added one ml of Ficoll reagent. the contents of the analyser cup were then carefully layered on to the top of the Ficoll, with a 'Pasteur' pipette. The tube was then centrifuged at 3,000 r.p.m. for 10 minutes. After centrifugation, it could be seen that the particles had sedimented according to their different density, with the white cells forming a band or zone about halfway from the top, and the red cells deposited at the bottom of the tube.

The white cell layer was then removed with a 'Pasteur' pipette to an all glass homogeniser. The cells were washed by adding 0.9% saline (2 ml) and centrifuged at 2,500 r.p.m. for 10 minutes. The saline was removed by suction, and the step repeated. Finally the remaining white cells were suspended in 0.5 ml of distilled water. These were then homogenised in an all glass homogeniser (Calbiochem Cat No. 150851) and centrifuged at 2,500 r.p.m. for 10 minutes. The supernatant (approximately 0.4 ml) could be frozen overnight at this stage.

— *Preparation of White Cell Smear.*

This was prepared from the white cell zone of a sample after gradient centrifugation, prior to homogenisation. A drop of the white cell suspension was spread on a clean slide, and dried in the air. This was stained in the Shandon Automatic Staining Machine, using the standard May-Grunwald Giemsa Stain.

— *Protein Procedure.*

The protein concentrations were determined using a manual method based on the method of Lowry[3]. The homogenate (0.1 ml), and 0.9% saline, (0.9 ml) were mixed, and to this was added 5 ml of freshly prepared alkaline copper tartrate and stood at room temperature for 10 minutes. Folin-Ciocalteau reagent (1 ml) was added, and the tube immediately mixed. After standing 30 minutes, the absorbance was read at 600 nm. A blank (1 ml of distilled water) and standard (1 ml of 0.1 mg protein/ml), were processed with the test. The protein concentration is given by:

$$\frac{A_T}{A_S} \times 0.1 \times 10 \text{ mg/ml},$$

where $A_T$ and $A_S$ are the absorbance of the test and standard corrected for blank absorbance.

**Results:**

The purity of the white cell preparation can be demonstrated in a stained film. The major component, apart from the leucocytes, is relatively large numbers of platelets. Very few erythrocytes are seen.

The protein concentration of several homogenates from different blood specimens is shown in Table II. This shows that a reasonably consistent recovery of protein is obtained from sample to sample.

TABLE II.—Protein Concentrations of Several Homogenates.

| Sample | Volume of Blood | Approximate Homogenate Volume. | Protein Concentration (in mg/ml) |
|---|---|---|---|
| 1 | 0.2 | 0.4 | 0.12 |
| 2 | 0.2 | 0.4 | 0.21 |
| 3 | 0.2 | 0.5 | 0.11 |
| 4 | 0.2 | 0.5 | 0.20 |
| 5 | 0.2 | 0.4 | 0.16 |
| 6 | 0.2 | 0.4 | 0.12 |

**Discussion:**

The diagnosis of Pompe's disease in the past required the collection of relatively large quantities of blood (10 ml) from very young, usually sick, infants. The method described shows how a sample suitable for enzyme assay can be obtained from a micro capillary sample of blood. The advantages of such a procedure

when screening numbers of children, a high proportion of whom have normal enzyme levels, are obvious.

The leucocyte preparation obtained is relatively free of contaminating red blood cells (the red blood cells are well below the usual ratio of 700 red blood cells to one white blood cell). Thus the enzyme determinations on the homogenate obtained from these cells will reflect the enzyme composition of the leucocytes. Contributions to the measured enzyme activity by contaminating cells would increase the apparent leucocyte $\alpha$-1, 4-glucosidase activity, and make interpretation of the results difficult. It is thought that although relatively large numbers of platelets are present, their mass, relative to the leucocytes, would be small. Work is proceeding to reduce the contamination by platelets.

The next step would be to verify that satisfactory enzyme measurement could be made. Using the methods described by Galjaard[1], preliminary experiments indicate that the method using p-nitrophenyl $\alpha$-D-glucopyranoside as a substrate would be sufficiently sensitive, using the micro homogenate described above.

A further study to identify the distribution of $\alpha$1, 4-glucosidase in different types of leucocytes is proposed, following development of the appropriate enzyme method. It is likely that a procedure based on that described by 'Pharmacia'[7], will give a good separation of the lymphocytes from the granuloctyes. The contribution to the observed enzyme activity from the platelets will also be evaluated using this procedure.

**Conclusion**

It has been shown that it is possible to obtain the leucocytes from as little as 200 microlitres of blood. Homogenates prepared from these cells can be used for determining the $\alpha$-1, 4-glucosidase activity of the leucocytes. This is particularly useful for the exclusion of Pompe's disease (Type II Glycogenosis) in small children presenting with enlarged hearts. Previously available techniques required the collection of 10 ml of blood, a difficult procedure with babies.

**Acknowledgments:**

To Dr C. W. Small and Mr I. Breed for assistance.

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# Technical Communications

## Particle Counters and Cold Agglutinins

After approximately 12 months of trouble-free red cell parameter counting and calculating we encountered recently a small technical problem affecting the results of a routine blood screen.

After initial dilutions of the E.D.T.A. sample in a modified Isoton Solution, the red cell parameters dilution was counted in a model ZF Coulter Counter, with flat-pack. Results obtained were

M.C.V. 156 fl
R.C.C. 1.6 x $10^9$/litre
Hct. 0.26

On looking at the stained blood film the red cells were seen to be clumped. Macroscopic examination of the E.D.T.A. sample and the initial sample dilution revealed agglutination of the red cells. Microscopic examination of the E.D.T.A. sample dilution showed gross agglutination.

On inquiring about storage of the blood before testing took place it was learned that the E.D.T.A. sample had been stored at 4°C for some six hours.

The sample was then warmed to 37°C in a waterbath and the red cell parameters repeated.

M.C.V. 86 fl
R.C.C. 4.7 x $10^9$/litre
Hct. 0.41

Antibody tests on the patient's blood revealed an auto-antibody with optimum reactivity at 4°C.

G. A. Erickson,
Nelson Public Hospital.

November, 1976.

## High Pressure Liquid Chromatography; Two Suggestions on Applications

High pressure liquid chromatography (HPLC) is becoming an increasingly popular system for compound separations and non-destructive analysis. Recently the opportunity to use the Philips LC-20 high pressure chromatography system arose, and during the operation two general points came to notice; one concerning solvent preparation and the other on sample preparation.

*Solvent Preparation*

It is essential when working with HPLC systems that the solvent systems be completely degassed. The recommended technique is heating the solvent under reflux. This technique requires constant observation and can involve considerable preparative work. An alternative solution to this problem is to degas the solvent under reduced pressure. The solvent is placed into a thick-walled, Erlenmeyer flask with a side vent; the top is bunged and the side vent is attached to a venturi pump. The pump is then run at full rate and the gas bubbles are seen to leave the solvent. When the gas bubbles cease to appear in the solvent, continue the reduced pressure for a further 10 minutes, after which time the solvent is ready for use. Solvents degassed in this manner were used up to and including 2,400 psi (16.7MPa) with no interference from dissolved gas. This technique cannot be used for degassing solvents with low boiling points.

*Interfering Compound in the Ultra Violet*

A number of manufacturers claim that native urine can be injected directly on to the HPLC column. This technique was tried without success. A broad peak was detected using a 254nm detector which did not correspond to any of the expected compounds being analysed. The fraction containing the peak was collected and scanned on a SP1800 recording spectro-photometer. The compound had absorption maxima at 234nm and 294nm. This corresponds to the absorption spectrum of uric acid. Uricase, 1mg/ml was added to the urine and incubated at 37°C for 30 minutes. After centrifugation, the urine was retested on the HPLC and the peak was absent, absorption spectrum of the appropriate fraction revealed no uric acid being present.

This emphasises the necessity for at least a simple sample clean-up step prior to injection on to the column as there are many UV absorbing compounds present in urine which could interfere with an assay.

M. Legge,
Christchurch Women's Hospital.

December, 1976.

# Abstracts of Submitted Papers NZIMLT Conference 1976

### $\beta_2$ Microglobulin and Renal Function

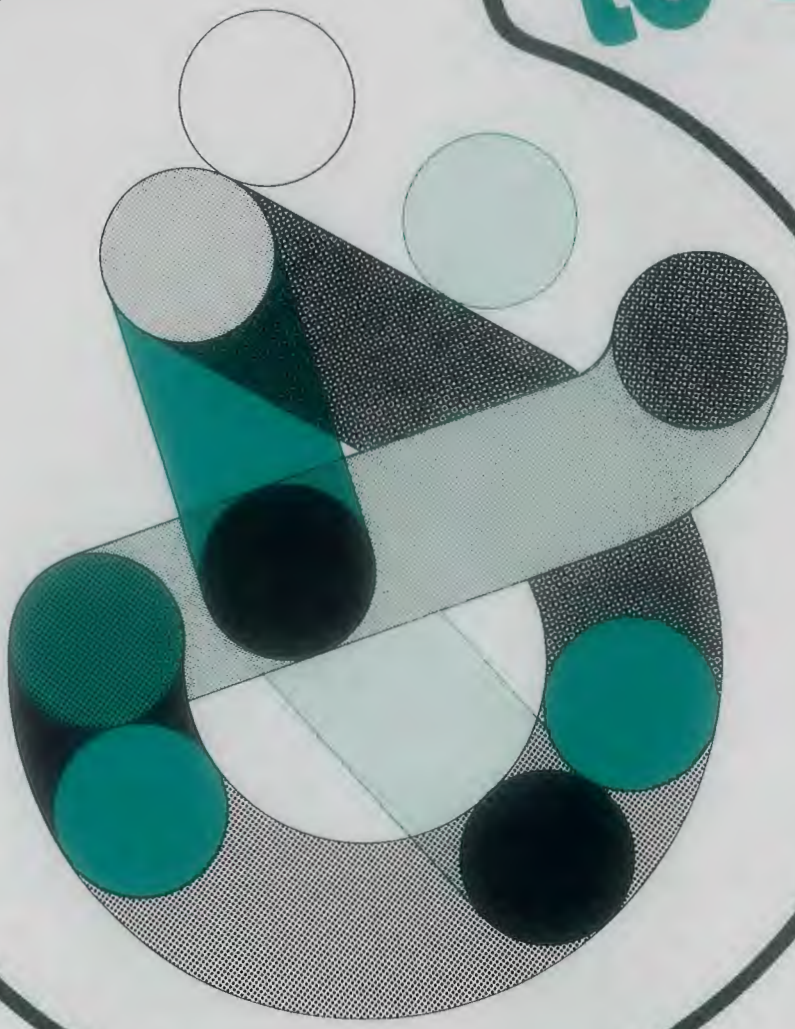
G. W. Tisch,
Clinical Biochemistry, Christchurch Hospital.

$\beta_2$ microglobulin is a small molecular weight polypeptide which may be found in relatively large amounts in the urine of patients with renal tubular disorders. The differentiation of glomerular and tubular proteinurias is one application for the assay of this protein. Most of it, after passing the glomerular membrane, is reabsorbed in the proximal tubules. Catabolism of $\beta_2$ microglobulin probably then occurs in the lysozymes.

Serum Creatinine and $\beta_2$ microglobulin correlate to a high degree and the latter is equally useful in measuring G.F.R. and the kidneys ability to reabsorb proteins in the proximal tubules.

# Fluka's here to stay

Fluka research chemicals have been invited back, again and again to hospitals and laboratories all over New Zealand. Fluka research and laboratory chemicals have set the standard. Orders are telexed weekly and delivered by air thirty days from receipt of an order.



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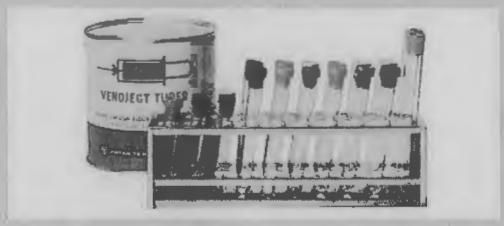


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$\beta_2$ microglobulin is conveniently quantitated by Radioimmunoassay in both serum and urine and a brief outline of this assay will be given.

Presented also will be some data derived from a small clinical study designed to evaluate the assay and determine 'normal' values.

**The Enzymatic Determination of Placental Function**

M. Legg, Perinatal Biochemistry Unit,
Christchurch Women's Hospital.

An enzymatic test of placental function, cystyl aminopeptidase, has been developed, and is in routine use in this laboratory. The test is rapid and simple to perform. Results correlate well with other tests of fetoplacental wellbeing and give results similar to Human Placental Lactogen.

Two versions of the method have been developed, a reaction rate technique and a colorimetric end point assay. Great care must be taken in the choice of the substrate, the merits of which will be discussed.

**Acetaldehyde, Fact or Fiction?**

K. G. Couchman,
Biochemistry Department, Massey University.

Attempts to measure acetaldehyde, an intermediate of ethanol metabolism, have been documented since 1920 with results ranging from 0.7 to 0.02 mmoles/litre. Present studies have shown the rapid disappearance of acetaldehyde added to whole blood samples. The mechanism of this reaction is not yet known but is associated with the presence of erythrocytes. The disappearance is sufficiently rapid for a major portion to be lost during venepuncture and subsequent centrifugation. Deproteinisation of the whole blood sample causes acetaldehyde to be produced in proportion to the ethanol concentration. By yet another mechanism, large amounts of acetaldehyde are produced from ethanol if haemolysis takes place. It may be a matter for speculation whether there are any other substances in the blood which behave in a similar manner.

**Quantitation of IgM by Electroimmunoassay**

T. R. Taylor and E. J. McKay,
Hamilton Medical Laboratory.

Electroimmunoassay (EIA) method for measuring Immunoglobulin M (IgM) has met with many technical problems in obtaining acceptable quantitative results.

As EIA has many advantages over radial immunodiffusion for routine protein quantitation, further comparative studies were undertaken to overcome the problems. This brief will consider results obtained from some of these studies which include carbamylation and sulphydryl reduction of the test plasma, and the use of various agar gels with differing electro-endosmotic flow rates.

**Investigation into Anti-placental Components**

M. Legge, Perinatal Biochemistry Unit,
Christchurch Women's Hospital.

The placenta is a unique organ, which exists in an equally unique situation. It appears to remain immunologically unscattered during pregnancy despite the production of a number of proteins, hormones and enzymes which are specific for the organ and are potentially antigenic.

Preliminary work carried out in this laboratory has been directed at detecting anti-placental components and investigating their significance during pregnancy. Results indicate that anti-placental components may be a factor in miscarriages and possibly diminished placental function.

**Variegate Porphyria**

Colleen Harrison,
Biochemistry, Waikato Hospital.

The body synthesises haem for the production of haemoglobin, cytochrome and myoglobin in the bone-marrow, spleen, liver, etc. Deficiencies in the enzymes catalysing the formation of the various precursors give rise to the porphyrias, which have only in recent years been clearly classified as: erythropoietic porphyria, erythrohepatic protoporphyria, acute intermittent porphyria, porphyria cutanea tarda, variegate porphyria and hereditary coproporphyria. In all the literature there are only five reports of variegate porphyria precipitated by oral contraceptives and no reports of variegate porphyria occurring in New Zealand.

This paper presents a case history of variegate porphyria probably precipitated by oral contraceptives. The fact that there have been no previous reports of variegate porphyria in New Zealand raises the questions of the mechanism of screening for porphyrias and the sensitivity of the screening tests. Comment will be sought on both screening and quantitative methods.

**Glycosuria in Pregnancy**

M. Legge, Perinatal Biochemistry Unit,
Christchurch Women's Hospital.

Over 50% of pregnant women develop glycosuria at some stage of their pregnancy, yet demonstrate normal glucose tolerance tests and no clinicopathological sequelae post partum.

Investigations into glycosuria during pregnancy have revealed there is considerable individual variation in glucose excretion and that this is dependent on a number of factors such as concentration, tubular reabsorption and gestation. It was also found that conventional glucose oxidase results are inaccurate in urine glucose analysis.

**A Mixed Drug Overdose**

T. E. Rollinson,
Biochemistry, Christchurch Hospital.

A patient was admitted unconscious with a possible mixed drug overdose. Gastric aspirate, urine and blood specimens were analysed for drugs. Diphenhydramine, methaqualone, paracetamol and a phenothiazine were found. Techniques used in the analysis were Thin Layer Chromatography, Ultraviolet Spectrophotometry and Gas Liquid Chromatography. This combination of techniques enabled unambiguous identification of all drugs present despite the screening effects of very intense reactions due to methaqualone.

**CK Isoenzymes**

D. Dohrman,
Biochemistry, Christchurch Hospital.

In the last few years there has been an increase in the demands for CK isoenzymes for the confirmation of A.M.I.

The massive MI does not require any enzymes for confirmation but the borderline and non MI can be elucidated faster by CK and LDH isoenzymes.

There are three main techniques for the separation and detection of the MB (heart fraction), these being, electrophoresis, chromatography, and activation. A review of these techniques is presented with some of our findings to date.

**Aspects of Bilirubin Protein Binding**

M. Killip, Laboratory,
National Women's Hospital.

It is well established that an excess of bilirubin over albumin which allows free bilirubin to diffuse readily into tissues is the fundamental cause of kernicterus. This paper will discuss current concepts of bilirubin distribution in blood and relate these to the bilirubin binding tests which have been proposed. The usefulness and limitations of various binding tests will be discussed.

**Two ABA-100, one ABA-100 plus one Beckman TR, or a Centrifichem?**

Ambil Darrah Laboratory,
New South Wales, Australia.

During the last few years there has been a rapid advance in new Clinical Chemistry Analysers. Often we are faced with difficult choice of choosing between instruments for our own particular situation. No instrument will do all the tests perfectly, but there are some tests that one particular instrument will do very well and others that it will not carry out as well.

The author of this paper has worked in a laboratory that purchased two ABA-100 and two Beckman TR's, and had six months experience setting these instruments up and finding the best chemistries to run on them. The author is now working in a laboratory that possesses a Centrifichem.

An evaluation of these instruments will be given and comparisons made.

**Evaluation of the Pye Unicam SP30 Reaction Rate System**

M. Legge, Perinatal Biochemistry Unit,
Christchurch Women's Hospital.

The Pye Unicam SP30 Reaction Rate System was evaluated for its suitability as a mid-priced dual beam reaction rate system. The instrument and printer performed well within the manufacturers' specifications. It was compared with methods from the Technicon Auto-Analyser Mark II and the Vitatron AKES; these results will be presented. The system was also assessed for ease of servicing, spare parts, appearance, spectrophotometric performance and optional extras.

**The Gilford 3500 Computer Directed Analyser**

Written by W. Hodgson; presented by R. McKenzie, Laboratory, Nelson Hospital.

The chemistry laboratory at Nelson Hospital (currently performing 1,200 tests per week) has, over the past 12 months, automated most of the routine portion of this workload with the Gilford 3500 Analyser. Instrument per-

formance has been very satisfactory and reliable and its routine operation is very quickly learned by all technical staff. This paper describes the basic operation of the analyser and will discuss its application in the laboratory (especially laboratories of similar size to Nelson) in terms of costs, range of applicable and adaptable methodologies and ease of maintenance.

**Gastrin — A New Diagnostic Aid in Gastroenterology**

G. W. Tisch,
Clinical Biochemistry, Christchurch Hospital.

The recent availability of Gastrin Radioimmunoassay reagents has placed this hormone in the service area of Clinical Biochemistry and it may now take a place amongst the diagnostic aids available to the gastroenterologist.

This paper will attempt to place gastrin in some perspective by briefly describing the chemistry, physiology, assay and clinical significance of serum gastrin levels in various pathological states. Some early findings will also be presented.

**Review of Recent Anaerobes Referred to N.Z. Health Institute**

D. Norris,
N.Z. Health Institute.

A brief description of the anaerobic organisms referred to the Reference Bacteriology Laboratory will be given. This will include the sites of isolation and the methods of identification. Isolates of two recently described species of *Bacteroides* will be included.

**Sensitivity Patterns of Bacteroides Species**

B. Cornere,
Microbiology Department, Greenlane Hospital.

The results of disc susceptibility testing for significant isolates of *Bacteroides species* are presented for the period of 1975 and the first six months of 1976. The testing method is briefly discussed and experiences with the V.P.I. Anaerobe Laboratory procedure are presented.

**Sensitivity of *Candida Albicans* to 5FC**

Sharon Vaughan,
Microbiology Department, Greenlane Hospital

Laboratory isolates of *Candida albicans* were tested for sensitivity to 5 fluorocytosine, using the standard strip sensitivity testing method on yeast morphology agar. Any isolates of doubtful sensitivity by this method were checked by doing a tube dilution M.I.C. Results will be forthcoming.

**Nutritionally Deficient Streptococci**

Mrs C. Smitheran,
Microbiology Department, Greenlane Hospital

Nutritionally defficient streptococci were isolated from Blood Culture of three patients—two with bacterial endocarditis and one with septicaemia. All were observed on direct smear but failed to grow on usual media under aerobic or anaerobic conditions. This paper will illustrate the type of medium used and the methods employed to isolate these fastidious streptococci.

**A Case of Failed Therapy**

Dr D. MacCulloch,
Greenlane Hospital.

A 43-year-old man was admitted with acute bacterial endocarditis. *Citrobacter diversus* susceptible to Cephalothin was isolated from Blood Cultures. This antibiotic was given in high doses but the patient failed to respond and required an aortic valve replacement eight days after admission. *Citrobacter diversus* was also isolated from the vegetations found on the aortic valve cusps at surgery, but this isolate was resistant to Cephalothin. Laboratory testing confirmed that the *Citrobacter diversus* from blood cultures and valve vegetations were similar except for Cephalothin susceptibility. The authors have been unable to find any previous reference to endocarditis due to this organism, or reports of resistance to Cephalothin developing in vivo.

**Identification of Human Staphylococci Using a Heat Stable Nuclease Test**

R. Menzies,
Microbiology Department, Greenlane Hospital.

Coagulase production by staphylococci is a marker of virulence. Production of coagulase, D'nase and thermostable D'nase were measured for staphylococci of human origin. Coagulase and thermostable D'nase production were found to correlate more closely than coagulase and D'nase production. The thermostable D'nase test is easier to perform than the coagulase test.

**Infection Due to *M. bovis* Strain B.C.G.**

R. H. Vaughan,
Microbiology Department, Greenlane Hospital

The use of B.C.G. vaccine results in occasional adverse reactions and infections. The

inoculation area aside, the most common site of infection is the axillary glands. Differention from *M. bovis* is difficult as all biochemical reactions are the same.

**Fungal Otitis Media — A Case History**

I. Wilkinson,
Microbiology Unit, Christchurch Hospital.

The patient was a 35-year-old male, with a clinical diagnosis of chronic suppurative otitis media. On five occasions a pleomorphic variant of *Aspergillus fumigatus* was isolated.

**Semi-automated Analysis of Blood Cultures**

Mr N. Wood,
Microbiology Department, Greenlane Hospital.

Two blood culturing systems are compared in parallel; a semi-automated radiometric method, BACTEC, and the conventional method used in our laboratory. BACTEC contains radioactive $^{14}C$ labelled substrates and monitors the level of $^{14}CO_2$ evolved by actively metabolising bacteria.

BACTEC was as efficient as the conventional method in detecting positive cultures and indicated that it was superior in detecting positive cultures earlier than the conventional system.

**Microbiological Data, Recording, Processing and Reporting Using an Optical Mark Reading System**

Mr C. Curtis,
Hamilton Medical Laboratory.

A urine recording and reporting system, utilising the I.B.M. 1232 Optical Mark Page Reader, I.B.M. 534 Card Punch and the I.C.L. 1260 computer is presented.

Advantages and possibilities of the system with reference to full microbiological reporting and subsequent data retrieval is discussed.

**A Futher Example of $Kp^a/K^o$ Exhibiting Depression of Some Kell Group Antigens**

D. S. Ford, A. E. Knight and F. Smith, to be presented by A. E. Knight.
Immunohaematology Department, Dunedin Hospital.

This paper gives details of a family in which there are numerous examples of the heterozygous $K^o$ state. The study was initiated by the presence of anti-$Kp^b$ in the serum of the propositus. Reactions with anti-k, anti-Ku, anti-$Js^b$ and anti-K13 confirm that the $K^o$ gene in *trans* with $Kp^a$ results in weaker expression of these Kell antigens. One example of anti-k showed some evidence of dosage effect with $kKp^b/K^o$ cells, but these results were unable to be confirmed by quantitation studies.

**Statistical Analysis of Genetic Typing in Paternity Disputes**

R. A. M. Anderson,
N.Z. Blood Transfusion Services.

The techniques used in genotyping the various genetic systems, are discussed and a statistical review of the last two years' work is presented. Genetical and practical problems encountered at various stages of the work will be examined and future developments extrapolated.

**Evaluation of RIA and PHA Techniques for $HB_sAg$ and $HB_sAb$ Detection**

R. A. M. Anderson,
Blood Transfusion Service, Auckland.

A report will be given of the comparative studies performed on one year's survey, between RIA and PHA techniques, utilised in $HB_sAg$ and $HB_sAb$ detection. The practical conclusions made from this study will be presented and also a short description of our current modifications being used, of the microhepatest technique for routine donor screening. Open discussion on any facet of the work will be welcomed.

**HBAg Testing: Macro or Micro?**

D. J. Haines and E. J. McKay,
Hamilton Medical Laboratory.

Since the introduction of commercial Haemagglutination Test kits for the detection of $HB_sAG$ modifications to reduce the cost per unit test have been applied to these kits.

This communication is intended to question the relationship between cost and sensitivity of the methods presently employed for $HB_sAg$ detection.

**Technique for In Vitro Culture of Haemopoietic Cells**

B. F. Postlewaight,
Haematology Department, Auckland Hospital.

The technique for the cultivation of haemopoietic cells in a semi-solid medium was introduced in 1965 using murine cells and in 1970 using human cells. This technique allows a measurement of the number of granulocytic progenitor cells and a certain proportion of the macrophage progenitors in any haemopoietic tissue. In this culture system, single haemopoietic cells can be stimulated to divide and form colonies. A substance termed 'colony stimulating factor' (C.S.F.) is required

# P.D. and How It Concerns You

**Michael Fitchett**
**Hamilton Medical Laboratory**

*Received for publication, July, 1976*

## What is P.D.?

Basically professional development (P.D.) means continuing education. Its definition must be broader than this, however, to cover a wide spectrum of technical and non-technical subjects which encompasses on-the-job training, self study, individual activities and formal training programmes both internal and external to a laboratory.

A fundamental characteristic of any learned profession is that its members must commit themselves to a lifetime of study. One's profession becomes a 'way of life' and is at the centre of a professional's thoughts and efforts almost continuously.

The dynamic nature of the medical laboratory profession's 'body of knowledge' is well known; it must not be portrayed as a fixed immutable inventory but rather as a foundation for what is expected to be an ever increasing store of learning, constantly augmented over the lifetime of a technologist. The intellectual habits of the technologist are assumed to be compatible with a creed of continual devotion to learning. Furthermore, the common body of knowledge itself will be neither static nor immutable but will advance to keep pace with the changes in the economic, social and scientific environment.

## Who is Responsible for P.D.?

Professional development is the responsibility of the individual, but much more support can and should come from his superiors. For the laboratory, the responsibility involves the provision of the environment, appropriate time and financial support and the working tools necessary to enable each member to develop to his top potential. There must be stress on self-development—a long term growth process as well as training programmes.

There is a certain degree of responsibility on the profession. The bodies acting as guardians of the technology must accept responsibility in areas of P.D. High standards of education, rapid technological development, growing complexity of clinical problems, all put pressure on the profession to assume a more prominent role in the professional development activity.

## Recognition of P.D. Activities

Steps must be taken to communicate to personnel that adequate recognition is given to P.D. activities including their own personal development and in the assistance of the development of others.

The importance of a good organised programme for the success of P.D. seems obvious. Such a programme, however, cannot exist unless there is enthusiastic active support from the profession and the individual laboratories both private and hospital.

P.D. cannot be instituted unless the environment exists. The costs involved would have to be recognised. Travel, programme organisation, personal expenses, and not forgetting the cost in time to those who prepare material and of the members who participate.

## Suggested Steps for Achieving a P.D. Programme

The initial impetus could come from the Institute of Medical Laboratory Technology. They could form a sub-committee to have P.D. as its goals and function.

The programme would initially examine the concepts of P.D. and all aspects pertaining to it, then determine the enthusiasm for such a programme amongst the members of the institute. The next task would be to determine the degree of responsibility that various organisations are willing to accept. These organisations are the Hospital Boards, the private laboratories, the N.Z. Society of Pathologists, N.Z. Association of Clinical Biochemists and any others.

For if any of these consider themselves as guardians of the profession then there must be acceptance of responsibility for P.D.

through every echelon of the members practising this technology.

**Conclusion**

The P.D. task is an enormous one that places heavy demands on the profession, not only in dollars but in time and talent, yet the profession's future is largely dependent on how well this task is performed.

P.D. is far more than an array of formal programmes, it is a concept and a framework that provides the environment and resources necessary for the lifetime growth of the professional man. Much of this framework can be provided by Laboratories and the regulating bodies, but the final desired result comes only from the dedication of each professional to his own development.

The difference between a technologist and a technician is only one of study, as defined in the Oxford dictionary. It might be said that once a technologist ceases to undertake P.D. then he becomes a technician.

In essence, P.D. exists to maintain and increase professional competence and, broadly speaking, a P.D. policy aims to do this by providing facilities for:

(a) Gaining competence in new techniques and developments

(b) Gaining knowledge in associated disciplines

(c) Enabling competence to be regained in areas of knowledge where there is now a deficiency because of a lack of recent experience.

It is within the experience of many, just how necessary these facilities are, to underestimate their importance is naive.

**The Trend Towards Compulsory P.D.**

There is a worldwide trend to compulsory P.D., not in the field of medical laboratory technology, but in other fields.

In the Accountancy Profession in the U.S.A. and Canada, P.D. is a mandatory requirement for an annual practising certificate in many states.

Within the Law Profession in the U.S.A., P.D. is mandatory in some areas.

In the Medical Profession, in the U.S.A., the Academy of General Practitioners requires its members to submit evidence of P.D. annually in order to retain membership. In Australia the Accountacy Profession are considering guidelines for possible introduction of P.D. policies.

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2. Roy, R. H. and MacNeill (1967), *Horizons for a Profession.* AICPA New York.
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# An Alternative Work Programme for a Medical Laboratory

**N. C. Paine, ANZIMLT**

**Valley Diagnostic Laboratory, Lower Hutt**

*Received for publication, September, 1976*

In the presence of a move to improve working conditions generally throughout New Zealand it was decided to attempt to apply an alternative work programme to this laboratory. This is a private medical laboratory covering a population of about 140,000. It is engaged in the collection of specimens from up to 70 km away and operated on a five days a week with all facilities open, as well as Saturday morning in Haematology and Bacteriology. There is almost no call back work carried out, thus no regular roster is required to cover this.

At a time when costs are rising, with limits on income, it was felt that a trial period on an alternative work programme would be one way of solving the problems facing private pathology and at the same time improve the conditions of work for those involved, as government controls on wages had caused a serious concertina effect on salary scales.

It was decided initially to research the the laboratory situation to determine the best possibilities open to a group of workers in plan. To this end a committee which comprised one member of each section (Chemical

Pathology, Bacteriology, Haematology, Serology, Office Staff and Nurses) was established. None of the members of this committee were heads of departments. This was deliberately done for two reasons:

(a) It gave other members of the staff a chance to have some say in the running of the organisation;

(b) It meant that none of the minor side issues in the management of the laboratory would cloud the thinking of the individuals in the group. Any problems which may not have been thought of were solved later.

This committee (which incidentally was all women) then produced a report detailing each department's ability to manage on present staff numbers, what is basically a four day week system. Variations to suit each department and individual desires were taken into account.

Each department was consulted after reading the report and a decision to take up the alternative work plan was made by the heads of departments and management, after consultation with the individuals in the department. This was a big decision as the laboratory was then committed to being open longer hours. Once an improved service was offered it had to be maintained.

However, because a move such as this may upset individuals each member of the staff was given the option of remaining with the old five day week or changing to the alternative. The reaction varied right across the board with one department completely changing, one department, with the exception of one member, doing a four day week, another with only one doing a shift system and the rest doing a five day system. The office had one person on a five day week, two on shift and two on a four day week. The nursing staff completely changed, except once every three weeks, two people had two half days instead of a complete day off. It is felt that this initial mixture of hours will gradually become more uniform.

The timing of the introduction is important. It was started one department at a time and the new service was offered once all departments were operative.

It was introduced at the end of winter and during the school holidays which is traditionally a slack period. This gave the staff a chance to adjust without the pressure of work being great.

One of the things which has helped in the smooth running of this alternative work programme has been that there is very little absenteeism due to sickness. For instance, the degree of sickness in chemical pathology department for three-quarters of the staff over a five year period is half a day per year per person and a couple of staff members have had seven and eight year stretches with no sick leave taken.

Annual leave is calculated on an hourly basis. In a week where there is an annual holiday such as Queen's Birthday and Labour Day then the remaining four days are worked on a seven and a half hour day, but staggered to provide cover over the extra period that the laboratory is open. All Saturday morning work in Bacteriology and Haematology is reimbursed as time off.

Those who are attending educational institutes are catered for in a variety of ways. Some have their day off on their lecture days, while trainees remain on the five day week and have time off as before. Variations within these extremes are allowed.

Basically then those who work the four day week work from 7.30 a.m. to 6 p.m. with an hour for lunch. One day each week is finished at 5.30 p.m. The day which each individual has off is a free choice provided no more than two people are away at one time. In practice this has not occurred very often.

A decision to have a day off may be made upon the morning of the day in question, but a warning that one could be taking a day off is given beforehand to the head of department. In practice, a priority list is placed in the department at the end of each week and staff indicate their choice for the next week on the list and it is worked on a 'first come first served' basis. This aspect of the change relies heavily on co-operation within each department. It goes without saying that days may not be stored up and used in another week. What are the advantages for the laboratory?

(a) The laboratory is open for longer to the general public. This allows people to come to the laboratory either before or

after work and the savings to the area in people not having time off work is immeasurable.

(b) No overtime is worked; a direct cost saving, and in an institution which relies on voluntary overtime it avoids the necessity of having to force people to do overtime.

(c) Reports, especially in bacteriology, are available sooner in the day, as are results on specimens which come in late.

(d) The ability to increase the number of different tests and the number of batches of a particular test within a day.

Technical staff has dropped to the same level as for 1969 and there is still room to cope with an increase in overall load. In the chemical pathology situation it was found that each batch of tests took about two hours. This is the total time from separation to reporting. Under the previous system this allowed one batch in the morning with time to spare and either one batch in the afternoon with a lot of time to spare or two batches with no time to spare. Now we have time for two batches in both the morning and afternoon with time to spare on each occasion.

This has eliminated the 'must be finished by 5 o'clock' syndrome which can lead to technical errors.

Personally I feel more relaxed while performing tests knowing that there is more time available.

As to the individual advantages, the day off is the big carrot and the flexibility to convert this to a four day weekend every fortnight is tempting. The more relaxed atmosphere at work is a benefit both to the laboratory and to the individual and the fact that there are two hours of the day when one's thoughts are not interrupted by the phone is sheer bliss.

The disadvantages are few. We contemplate a few problems over the Christmas holiday period but nothing insurmountable. There is also an increase in the use of power, gas and services because they are used for a longer period. However, we would expect this to be minimal and certainly offset by the decrease in wage payments for overtime. To the individuals the thought of getting up earlier and home later probably upsets those with families more than single people. Although it certainly puts more strain on wives at home with children like a lot of things it is a matter of adaptation and the new hours soon become moulded into the family life. There is a loss of overtime payments but in our case this only affected three or four individuals.

I believe that there is a growing appreciation of the advantages of this approach to the working week and hope that this brief description of our experience will be of benefit to others contemplating such a move.

## Correspondence

Sir, — It was most interesting to read the two articles on creatine kinase (CK) assays in *N.Z.J. med Lab Technol.* **30**, 3 (1976), by Dohrman, and Lane *et al.* respectively. In view of the two papers I would like to point out some of the problems associated with total CK and CK isoenzyme assays.

(1) At 37°C CK isoenzymes will react differently to a common substrate. After five minutes incubation at 37°C (with or without substrate) the following approximate loss in enzyme activity is found. MM 12%, MB 10%, BB 25%. The addition of bovine serum albumin will not prevent the loss in activity, indeed with BB it enhances loss of enzyme activity.

(2) Incubation with dithiothreitol at 37°C results in an enzyme activity loss after five minutes of MM 12%, MB 3%, BB 5%. In this case the addition of bovine serum albumin will not affect MM or MB activity any further, however, BB activity rapidly deteriorates by approximately 60% of the original activity.

(3) The nature and concentration of the CK activator, $NADP^+$ and ADP/AMP concentrations are all vital in the CK assay. For example reduced glutathione may well oxidise which could in turn react with NADPH, the product of the CK reaction, producing low CK results. Too high a $NADP^+$ concentration also inhibits CK isoenzyme reactivity particularly when using electrophoresis to identify CK isoenzymes. With high reaction product concentrations (NADPH) the CK reaction deviates

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Specific : detects five related streptococcal enzyme antibodies* and is unaffected by nonspecific enzyme inhibitors. (2)
Stable : may be stored for over 1 year at refrigerator temperature.

Streptozyme TM Kits for 15 tests, including positive and negative controls

* ASO, AH, ASK, ADNase, ANADase


(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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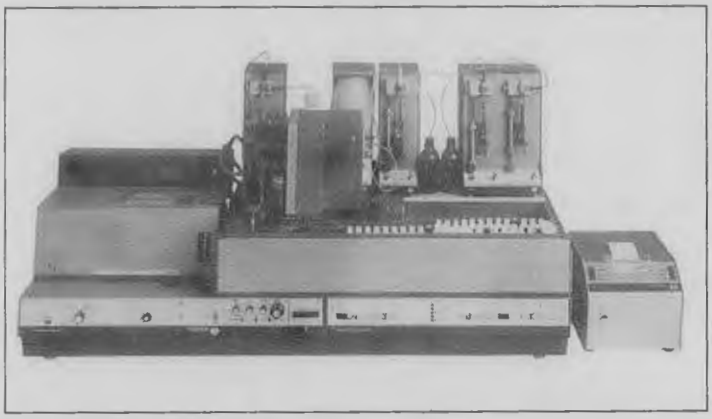

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from zero order kinetics, this will occur with high CK activity.

(4) CK isoenzymes are also inactivated by urea and uric acid, possibly involving the sulphydryl groups of the enzyme. This is completely reversed by the addition of thiol reagents.

(5) There is also a species difference in terms of reactivity of CK isoenzymes, e.g., rabbit MM and BB are unstable particularly after dilution, but chicken MM and BB are considerably more stable.

(6) The three isoenzymes have differing kinetic properties, i.e., BB shows a higher activity than MM at lower substrate concentrations, while increasing substrate concentrations cause more inhibition of BB. The MB isoenzyme is intermediate between BB and MM in kinetic properties. Maximal difference of MM and MB activity occurs at pH 7.5 and not at pH 7.0.

(7) A fourth CK isoenzyme exists which is thought to be unique to the mitochondria of the heart. This has different kinetic properties to the other three CK isoenzymes.

(8) With the possible exception of the mitochondrial CK isoenzyme no CK isoenzyme is unique to a specific organ. MM is found in various muscles beside skeletal it is also located in brain, lung, thyroid, prostate, kidney, heart, small intestine and spleen. MB is found in various muscles including skeletal and is also located in intestine, heart and prostate, and BB is found in brain, lung, thyroid, small intestine, bladder muscle and prostate.

These problems associated with CK assays have obvious implications in diagnostic laboratory work. First, the temperature of the assay is critical 30°C is better than 37°C and 25°C is better than either. Bearing in mind the CK reaction has a lag period of approximately five minutes the loss in enzyme activity due to temperature is important. Dithiothreitol helps to prevent this loss of activity, however, the presence of protein may negate dithiothreital effect particularly in the case of BB activity.

The effect of CK activator on the assay is well documented, but secondary reactions taking place during the actual CK reaction is not fully realised. Inhibition of CK activity by uric acid and urea is probably not generally realised. Correlation of electrophoresis CK isoenzyme studies, which are usually performed at 37°C and use high $NADP^+$, with chemical assays must be viewed with some scepticism.

The use of commercial CK control sera is also a point which carries a cautionary message. Different manufacturers use different species to supplement their CK value, particularly if the CK values are elevated. This can lead to two, or more, types of CK isoenzymes in the control sera with differing kinetic properties, and stability, thereby giving a variety of results dependent upon dilution, time of reconstitution and nature of other constituents.

Whilst the MB isoenzyme is a predominant CK isoenzyme of heart, the heart also contains MM and the mitochondrial CK isoenzyme. Each of these can only be assayed using specific conditions such as optional pH, substrate concentration, cofactor requirements and buffer species.

The message is, therefore, that diagnostic differential CK isoenzymes are difficult to assay, however, there is now sufficient evidence to support the use of CK, MB in the differential diagnosis of myocardial infarction. The release of the isoenzymes MM and MB after myocardial infarction may well depend upon the extent of damage and where the damage has occurred in the heart. Finally the release of the mitochondrial CK isoenzyme would be the isoenzyme of choice to assess cardiac damage and its presence in the serum could possibly be a factor in assay anomalies seen in the differential CK isoenzyme assessments of myocardial infarction.

M. Legge,
Perinatal Biochemistry Unit,
Christchurch Women's Hospital.

Sir, — We refer to the Technical Communication on Creatine Kinase Isoenzymes provided by Lane *et al.*, New Zealand, N.Z.J. Med. Lab. Technol. November, 1976.

The basis of the selective activation method of Rao *et al.* (1975) varies from that stated in their paper. Their statement reads:

"The Calbiochem CPK/MB kit is designed to measure total creatine phosphokinase activity—the activity found in the presence of a sulphydryl activator and also the MM fraction —the activity in the absence of activator." Rao promulgates that the isoenzymes should be

measured utilising the differential activating capacities of two different sulphydryl compounds. The MM activity is actually measured in the presence of GSH, while the total is measured in the presence of GSH plus DTT.

The reagent is not measuring the difference between a completely inactivated CPK and an activated CPK, as implied by the authors. The difference that would be seen in such assays would always be present and would be of an order of magnitude of one half the activated level.

The curves supplied by the authors are interesting. By superimposing the two MM and the two Total curves, fluctuation seen in the MB fraction with the Calbiochem method can be seen to arise from the fluctuation of the totals, since the MM curves superimposed virtually identically. The resulting values for MB fluctuate between —180 to +200 mU/ml. Such large negative numbers may reflect imprecision in assay technique since normal levels of MB range only up to 15 mU/ml at 30°C.

The values obtained considerably exceed the levels of linearity of the reagent and it would be interesting to know assays detail, especially whether the dilutions were made before or after the samples were activated by the DTT. The instrumentation and reagents employed in conjunction with the glass bead technique have not been described for comparative purposes.

We consider the authors to be correct in their conclusion that lower activity will be found using a non-reactivating reagent but in this case it does not apply.

N. G. Sutherland,
Calbiochem Asia-Pacific Regional Headquarters,
Hong Kong.

## Abstracts of Technical Projects in Par tial Fulfilment of the Examination of Qualified Technical Officer.

**A Simplified Quantitative Method for Lactic Dehydrogenase Isoenzymes Levels as part of a Cardiopulmonary Bypass Survey.** Mrs Karen Hubbert, Hamilton Medical Laboratory, February, 1976.

A survey was made on patients undergoing cardiopulmonary bypass operations both clinically and enzymatically for one day preoperatively and three days post-operatively by the heart unit physicians. The project describes the protocol used and the modification of an LDH isoenzyme method developed to cope with up to nine patients a week. Suitable methods of storage were also investigated.

The project was based on a study undertaken at Green Lane Hospital Laboratory in 1972.

**The Value of Monitoring Human Chorionic Gonadotrophin Levels in Trophoblastic Disease.** Robyn E. McCulloch, Postgraduate School of Obstetrics and Gynaecology, National Women's Hospital, Auckland, March, 1976.

Plasma and urine human chorionic gonadotrophins levels were measured to evaluate their use in monitoring patients with hydatidiform mole and choriocarcinoma. HCG in urine was measured using a direct agglutination immunoassay and the plasma HCG was measured using a solid phase radioimmunoassay. The data was collected and assayed as the patients became available. The information found provided the average disappearance rate of HCG from the patient's circulation following evacuation of molar tissue. From this appraisal it would seem unnecessary to do plasma estimations on patients with high levels of HCG, however at low levels both urine and plasma estimations are preferable.

**The Effect of Blood Donation on Platelet Function.** Sandra A. Swann, Blood Transfusion Centre, Auckland, February, 1976.

The Study was undertaken primarily to establish whether any change occurs in *in vitro* platelet function after blood donation. Twenty donors were tested pre and post donation and the platelet concentrate tested during storage. Thirty additional donors were tested post donation only. As was expected there was no significant platelet function changes before and after blood donation. Reduced viability or impaired haemostatic function appeared to occur as a result of centrifugation or storage procedures.

# Obituary

## NORMAN JOSEPH ELLISON.

Norman Joseph Ellison died suddenly at his home on November 30, 1976. He was a Life Member of the New Zealand Institute of Medical Technology and Principal Technologist at the Medical Laboratory, Wellington.

Mr Ellison, affectionately known as Tom to his many friends, was to retire early in 1977 and after many years spent in laboratory work and in endeavour on behalf of the New Zealand Association of Bacteriologists, as the Institute was formerly known, his planned retirement was well earned.

He was educated at Auckland Grammar School and graduated B.Sc. from Auckland University. His introduction to a medical laboratory was at Auckland Hospital where he trained under the late Dr Walter Gilmour. After qualifying he spent two years at the Dairy Research Institute in Palmerston North before taking the position of Charge Bacteriologist at Napier Hospital. So highly was he regarded in this position that he was elected an Honorary Member of the local Medical Association. His next and final move was to Wellington where he joined Dr P. P. Lynch's laboratory as Principal Technologist, a position he held for the next 40 years.

A foundation member of the New Zealand Association of Bacteriologists, Mr Ellison was the second President from 1948 to 1950. It is widely acknowledged that the Institute owes him an immense debt for his work on the construction of the Association on permanent lines. At the first conference in 1945 the draft constitution for an Incorporated Society which had been compiled by Mr Ellison was submitted and approved. During his Presidency an agreement was made on the syllabus for the Final Examination and the long awaited Intermediate Examination—the forerunner of the present Basic Training Part I Examination—was approved in principle.

Much of Mr Ellison's work for the Association was connected with its legal proceedings. In 1949 there was an attempt by an industrial union to include certain laboratory staff in that union and the Association joined in proceedings in the Supreme Court to oppose this. It is to Mr Ellison's credit that the eventual outcome was satisfactory to the Association.

Such was the appreciation of the value of Mr Ellison's contribution as a member of the Association Executive that Life Membership was conferred on him in 1951.

While at school Mr Ellison was a proficient hockey player; in later life, his greatest interest outside his work was in golf. He was a very good player with a profound knowledge of the game. He had an outstanding career in golf management, being a committee member of the Manawatu Golf Club, a committee member, Club Captain and President of the Hutt Golf Club which elected him a Life Member in 1974. He was a member of the New Zealand Golf Council and Vice-President of the New Zealand Golf Association for almost 30 years.

Mr Ellison was meticulous of habit. Disorder was anathema to him, and his orderly mind allowed him to see things in their proper perspective. He enjoyed an extremely good relationship with his colleagues and the many problems referred to him were testimony to the high regard in which his opinions were held. He had the respect of all and his loss is keenly felt.

Mr Ellison is survived by his wife, two daughters and son, to whom we offer our deepest sympathy. A. L. S.

# Abstracts

Contributors: E. R. Crutch, J. Hannan and Janice Parker.

## CLINICAL BIOCHEMISTRY

**Evaluation of a New Enzymatic Kit for Serum Triglycerides.** Carter, T., Martin, P. J., Wilding, P. and Goldberg, D. M. (1976), *Clin. Chem.* 22, 520.

A new enzymatic kit for serum triglycerides is evaluated. The procedure involves enzymatic hydrolysis of the triglycerides and conversion of the glycerol, which is linked to an indicator reaction. The test requires 50 μl of serum and can be completed in about 50 minutes. Results are compared with those of the mechanised fluorometric method and with manual procedures. The method was found to be satisfactory, inexpensive and simple to perform. —J. P.

**Normative Values of Serum Immunoglobulins, by Single Radical Immunodiffusion. A Review.** Maddison, E. E. and Reimer, C. B. (1976), *Clin. Chem.* **22,** 594.

The authors present a review and discussion of normative values of IgG, IgA and IgM in human serum from adults with brief comments on the findings in children and normative values for IgD and IgE. Immunoglobulin concentrations were found to reflect race, age, sex and environment and vary significantly in subpopulations of man. —J. P.

**Rapid Radioimmunoassay of Total Urinary Estriol.** Anderson, D. W. and Goebelsmann, U. (1976), *Clin. Chem.* 22, 611.

A specific and reliable method for total urinary estriol conjugates is presented. A 25 μl aliquot of the urine is acid hydrolysed, neutralised, and diluted before assay. Using semi-automatic pipetting equipment, one laboratory technologist can complete 50 assays within eight hours. —J. P.

**Clinical Laboratory Evaluation of the Perkin-Elmer KA-150 Enzyme Analyser.** Hardin, E., Passey, R. B., Gillum, R. L., Fuller, J. B. and Lawrence, D. (1976), *Clin. Chem.* 22, 434.

The Perkin Elmer KA-150 Enzyme Analyser was used in parallel with the laboratory's routine methods for five commonly assayed enzymes and evaluated for rate of analysis, linearity, precision, carryover and error detection. The machine uses classic two reagent determinations and was found to be dependable, rapid, precise and simple to use. —J. P.

**A New Method for the Determination of Serum Creatinine Based on Reaction with 3-5 Dinitrobenzoyl Chloride in an Organic Medium.** Parekh, A. C., Cook, S., Sims, C. and Jung, D. H. (1976), *Clin. Chim. Acta* **73,** 221.

The commonly used Jaffe alkaline picrate reaction for creatinine in serum and urine commonly estimates as much as 30% non-creatinine chromogenic material. The use of DNBCl in an organic medium gives a creatinine procedure of improved sensitivity and linearity. The authors recommend the proposed method for routine clinical use on the basis of data submitted. —J. P.

## Haematology

**The Serum Vitamin $B_{12}$ Level: Its Assay and Significance.** Mollin O. L., Anderson, B. B. and Burman, J. F. (1976), *Clinics in Haematology* 5, 521.

Principles and comparison of *Euglena gracilis, Lactobacillus leichmanii* and radio-isotope dilution assays of Vitamin $B_{12}$. The significance of low serum $B_{12}$ levels by each of these methods is discussed including iatrogenically falsely low levels. —E. R. C.

**Investigation and Management of Megaloblastic Anaemia.** Chanarin, I. (1976), *Clinics in Haematology* 5, 747.

Discussion on diagnosis of Megaloblastic Anaemias including mean cell volume, peripheral blood film, bone marrow examination, serum assays of $B_{12}$ and folate, and absorption tests. The article concludes with a short discussion on therapy and management. —E. R. C.

**Preparation of Concentrated Platelet Suspensions by Dehydration against Polyethyleneglycol 20,000.** Akkerman, J. W. N., Gorter, G., Webster, J. and Sixma, J. J. (1976) *Scand. J. Haematol.* **17,** 71.

Gel filtered platelet suspensions were concentrated by repeated dehydration against polyethyleneglycol 20,000 followed by dialysis against slightly hypotonic buffer. This method increased the platelet concentration 2-3 times with a recovery of 80-100%. The final cell suspension closely resembled the original platelet rich plasma as tested by a number of platelet tests. —E. R. C.

**On the Value of the Activated Partial Thromboplastin Time (APTT) in Monitoring Heparin Therapy.** Teien, A. N., and Abildgaard, U. (1976), *Thrombos. Haemostos.* (*Stuttg.*) **35,** 592.

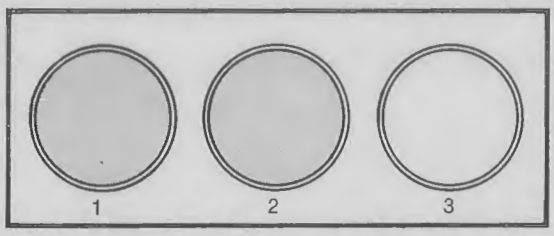
Of 1,068 blood samples referred for evaluation of the haemostatic mechanism 135 contained heparin as judged by the polybrene titration method. Of the 57 samples with a heparin concentration in the defined therapeutic range, 65% had APTT values in the "therapeutic range". Of the samples from patients who had not received any anticoagulant, 24% had prolonged APTT, and 48 of these samples had APTT values in the "therapeutic range" for heparin therapy. It is suggested that the APTT test is of limited value in monitoring heparin therapy. —E. R. C.

**Comparative Sensitivity of Two Haemagglutination Inhibition Immunoassays for Fibrinolytic Degradation Products in Human Serum.** Chi, D. S., and Chen, J. P. (1976), *Thrombos. Haemostas.* (*Stuttg.*), **35,** 423.

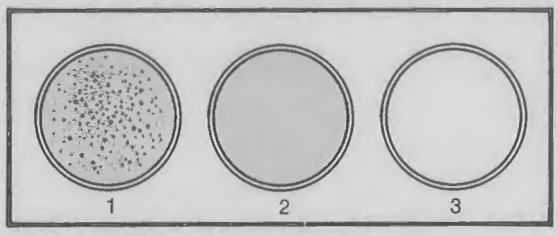
A new modification of haemagglutination inhibition immunoassay for measuring fibrinogen/fibrin degredation products using glutaraldehyde as a coupling reagent was introduced. This method was compared with the tanned-red-cell haemagglutination inhibition immunoassay. —E. R. C.

*IN INFECTIOUS MONONUCLEOSIS TESTS . . .*

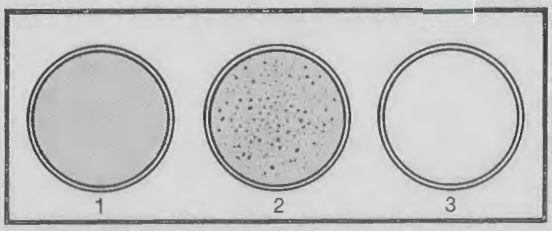
# what's the diff?



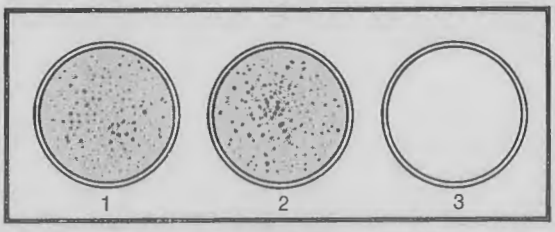

No agglutination in either test circle: **negative** for Infectious Mononucleosis. Absence of agglutination in both circles can also indicate a **positive** test for serum sickness antibody, since both absorbents neutralize this antibody. To confirm the presence of serum sickness antibody, test one drop of patient sample with one drop of MONO-DIFF TEST Reagent, without absorbents. If agglutination is observed, the test is **positive** for serum sickness.




Stronger agglutination in circle 1 than circle 2:
**positive** for Infectious Mononucleosis.




Stronger agglutination in circle 2 than circle 1:
**positive** for Forssman antibodies.
**negative** for Infectious Mononucleosis.




Equal agglutination in circle 1 and 2:
dilute patient sample 1:20 and repeat test.

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- Confers greater ease and readability, adds unique stability (one year shelf life) to a differential test;
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**New Instrument for Automatic Recording of Clot Lysis.** Wilkens, H. J. and Back, N. (1976), *Am. J. Clin. Pathol.*, 66, 124.

An experimental instrument that determines and automatically records the end-point of clot lysis is described. The instrument is unique in that it measures the end product of lysis directly and quantitatively, unlike currently available indirect methods that rely on optical or mechanical changes indicative of lysis. —E. R. C.

**The Reticulocyte. An Approach to Definition.** Gilmer, P. R., and Koepke, J. A. (1976), *Am. J. Clin. Pathol.*, 66 (*Supplement*) 262.

Experiences of the CAP Survey Programme in reticulocyte counts and morphologic identification of reticulocytes from 1971 to 1974 are reviewed. Problems of morphologic identification are reflected in an excessive variance of reticulocyte counts. —E. R. C.

**Serum Folate Levels. Comparison of Microbiologic Assay and Radioisotope Kit Methods.** Waddell, Caroline C., Domstad, Peggy A. Pircher, F. J., Lerner, S. R., Brown, J. A. and Lawhorn, Beverly K. (1976) *Am. J. Clin. Pathol.* 66, 746.

Serum folate levels were measured prospectively in 72 patients and six normal controls using *Lactobacillus casei* microbiologic method and a commercially available sequential-binding radioassay kit method. The authors found that these commercially available radioassay kits yield reliable values and can be readily performed in a clinical radioisotope laboratory. —E. R. C.

## MISCELLANEOUS

**Recent Progress in Automatic Image Analysis Instrumentation.** Hougardy, H. P. (1976), *Microscope*, 24, 7.

The principles of automatic and interactive pattern recognition are summarised which form the background in the assessment of the performance properties of modern image analysis instrumentation. This is followed by a presentation of the salient features of new instruments and, in the case of the better known image analysers, the new modules available. The paper concludes that further improvement depends on the customers and manufacturers, individually as well as on their collaboration. (Author's Abstract). —J. H.

**Cytomorphologic Changes in the Leukocytes of Patients with Malignant Disease.** Johnston, B. and Brady, J. M. (1977), *Lab. Med.* 8 (1), 28.

The authors, at the present time, do not recommend their observations as a general screening test for cancer or for a specific type of malignant neoplasm because of insufficient data involving blood cytology. However, the changes can be used in the management of patients undergoing treatment. The cytomorphologic changes described consist of (a) thin, threadlike nuclear excrescences protruding from the inner aspect of polymorphonuclear neutrophils in 20% or more of the polymorphonuclears and (b) small blue inclusion bodies surrounded by lightly stained areas, or 'halos', in the cytoplasm of the large mononuclear cells in 50% or more of the large mononuclears.

In a series of 2,503 patients one observer was 89% correct in differentiating cancer patients from noncancer patients while a second observer was 90% correct in the same series. It is felt that a six to 12-month training period is required to obtain similar results. J. H.

**The Identification of Accelerated Coagulability.** Caprini, J. A., Zuckerman, L., Cohen, E., Vagher, J. P. and Lipp, Virginia. (1976), *Thromb. Res.* 9, 167.

Native whole blood thrombelastography and celite activated thrombelastography were performed on the same blood collected from 90 normal individuals and 90 patients with new malignancies and the results used to derive a discriminate equation. This equation classified correctly all 90 normal individuals and 88 of the cancer patients. Identification of accelerated coagulability in asymptomatic populations may identify occult problems, including cancer. —J. H.

# Book Reviews

**Review of Medical Microbiology.** Twelfth Edition. Ernest Jawetz, Joseph L. Melnick and Edward A. Adelberg. Published by Lange Medical Publications Los Altos, California, 1976. Price $NZ12.00. 523 pages, illustrated. Obtained from N. M. Peryer Ltd., Christchurch.

This book, popularly known as "Jawetz" should need no introduction to medical microbiology workers. Once again the authors of this twelfth edition have been able to give a concise and readable account of virtually all aspects of medical microbiology.

Two hundred of the 523 pages of text are devoted to virology and in this section notable additions include rotavirus (orbivirus) and Nowalk agent both likely causes of sporadic and epidemic non-bacterial gastro-enteritis, Marburg virus and brief accounts on the relationship of viruses to diabetes mellitus and Crohn's disease. Recent advances in hepatitis research are included. One of the many assets of "Jawetz" is this precise summary of viruses and virus induced diseases.

The chapters on cell structure, physical properties and the genetics of bacteria are lucidly presented. Technologists and other staff involved in diagnostic medical microbiology will benefit from a knowledge of these aspects of bacteriology and thus find that

reading the appropriate parts in this book a useful background to their work.

No doubt, the 30 pages devoted to parasitology are not as colourful and detailed as specialist texts but they are a useful and practical summary of this subject.

Some notable defects occur in the chapter on anti-microbial chemotherapy. Although the more recently described cephalosporins and tetracyclines are mentioned nothing is stated on the aminoglycosides tobramycin and amikacin. In the table on drug selection for various infections the first choice antibiotic for *B. fragilis* infection is correctly given as clindamycin but penicillin is listed as one of the alternative drugs. *B. fragilis* is almost always highly resistant to penicillin. The suggestion that polymyxin is an alternative first choice antibiotic in pseudomonas infections is not valid in 1976.

The text was originally designed for medical students and practising physicians and since the biannual reviews always include new work and knowledge, the book maintains its usefulness to these people.

Although hospital laboratory libraries may not wish to have every new edition, the possession of a relatively recent copy of "Review of Medical Microbiology" is always a most handy reference to have available.

E. R. Smith.

**Fundamental Skills in Serology,** by Leila J. Walker and Howard Tubb. An Allied Health Professions Project 1976. Published by Charles C. Thomas, Springfield, Illinois. Price $US14.95. 473 pages, illustrated.

This text considers some of the more simple serological procedures undertaken in medical laboratories. The authors state this instruction manual can be used as a general introduction to the field of immunology for students and instructors alike.

Each procedure is written in modular form with a sketchy outline of the theory surrounding the text, a very detailed step by step illustrated protocol, a list of relevant definitions, a self evaluation and an instructor's performance checklist.

The book is divided into 15 separate modules dealing with a chapter on Dilution techniques; a chapter on Agglutination Tests using latex particles, red cells and bacterial suspensions; a chapter on the FTA-ABS and a chapter on Quality Control. It seems a pity that Complement Fixation, an important and more common serological procedure, has not been considered.

The test procedures utilise known commercial antigenic preparations such as the UCG test for pregnancy or the Hyland CR latex reagent for the detection of C-Reactive Protein when describing both theoretical and practical concepts of the various tests.

The theoretical aspects of each test procedure appear to be little more than what appears on the manufacturers' instruction inserts whereas the technical detail given for the various modules is unnecessarily lengthy and rather boring, for example the authors have taken nearly 40 pages to describe and illustrate the simple procedure of 2, 5 and 10 fold dilution steps.

Each module is liberally illustrated with "humorous" sketches throughout dealing with "dos and don'ts" of each test procedure which in the reviewer's opinion are generally unnecessary. The book has been made in a ring binder form and protected with a heavy plastic cover so it may be used as a bench manual.

The concept of teach-learn and self examine is good, however, because of its bulk and rather excessive monologue this book will probably have limited appeal in New Zealand medical laboratories for other texts currently in use for medical technology training cope with this area of Immunology in a more concise and realistic approach.

E. J. McKay.

**Analytica Chimica Acta, Computer Techniques and Optimisation** will be published quarterly, four issues per year constituting one volume. The first issue is scheduled to appear in March, 1977.

SUBSCRIPTION INFORMATION

The new section will form an integral part of Analytica Chimica Acta, which will continue under the general editorship of Professor Philip W. West and Dr A. M. G. Macdonald. However, it can be subscribed to separately. The subscription price for the first volume is $US49.75/Dfl. 129.00, including postage.

Free sample copies will be available on request from the publishers: Elsevier Scientific Publishing Company, P.O. Box 211, Amsterdam, The Netherlands.

AIMS AND SCOPE

The new section of the journal will publish papers dealing with:

: : Computer acquisition, processing and evaluation of data.
: : Computer methods for the interpretation of analytical data including chemometrics, cluster analysis and pattern recognition.
: : Storage and retrieval systems.
: : Optimisation procedures and their application.
: : Automated analysis for industrial process and quality control.
: : Organisational problems.

TYPE OF CONTRIBUTIONS

The contributions will generally emphasise any of the aspects specified above. Formally, the following types of manuscripts will be accepted:

: : Regular papers.
: : Short communications.
: : Reviews (reviews are written by invitation of the editors, who welcome suggestions for subjects).
: : Information regarding new instrumentation, forthcoming events, new literature.
: : Reviews of books within the scope of the journal.

The language of the journal is English, although papers may also be submitted in French or German.

Fifty reprints will be supplied free of charge.

Instructions to authors are available from the publishers on request.

Manuscripts should be submitted to Dr J. T. Clerc (address as above).

**Introduction to Medical Sciences for Clinical Practice. Unit I: The Cell Concept.** Multiple authorship. First edition 1976. Published by Year Book Medical Publishers, Inc. 389 pages, illustrated. Prize $NZ11.40. Supplied by N. M. Peryer Ltd., Christchurch.

This volume is the first in a series of 15 intended as a self instructional tutorial curriculum for physicians' assistants. The book lacks a preface to explain its application but one can infer that it is the required text for the Physicians' Assistant Program, the Bowman Gray School of Medicine of Wake University, Winston-Salem, North Carolina. Physicians' Assistants, a North American innovation, are presumably able to obtain certification through this programme. The text is divided into five modules covering chemical composition of cells, the cell, energy and cellular metabolism, protein synthesis and finally electrical properties of cells. Each module is then further subdivided into mini-courses containing objects to learn, study material and questions to work through.

The book is interspersed throughout with comic-book type illustrations, some of which serve to clarify the issue. Some neither amuse nor clarify and I felt several sections could have been better explained in simple chart form. Many people may prefer a more formal approach.

The analogies in the section on the Nerve Impulse are particularly difficult to follow and I suspect a student with no previous knowledge of the topic would emerge feeling very confused. Several typographical errors occur which could be misleading to the uninitiated, e.g. menophilia for hemophilia and Hcl for HCl.

Despite this criticism of format the material presented is clear and concise with excellent summaries. The biochemistry sections are particularly well covered. Used in the manner intended, as an actual study course in itself, a sound basic knowledge of the subjects presented would certainly be obtained. However, the course is better suited for a student of basic biology and I would not regard it as a substitute for the basic texts used in the New Zealand Certificate of Science (Paramedical) Course.

J. Parker.

**Mathematical Preparation for the Health Services.** 2nd Ed. 1976, by Joseph Routh. Published by W. B. Saunders Co., and supplied by N. M. Peryer Ltd., Christchurch. 157 pages. Price $NZ7.15.

If you suffer from doubts about your mathematical knowledge and ability to benefit from a text book on the subject this book might well dispel them. Few assumptions of previous knowledge are made. The initial discourse is on handling numbers, fractions and decimals and the author then goes on to explain logarithms and the use of the slide rule. (A dissertation on the bench calculator which seems to have largely superseded the slide rule would not have been out of place.) Simple treatment of pH, and hydrogen ion

concentration and buffers follow. These chapters are followed by a few worked examples and some exercises. Common laboratory calculations such as are involved in preparing solutions, renal clearances, calculations and graphs have separate chapters devoted to them. The spectrophotometric calculations exemplified are perhaps rather more simple than the type which has appeared in our final examination papers.

Quality Control covers the basic calculations of standard deviation, the construction of quality control charts, the use of the chi-square in instrument control, the use of graphs, types of laboratory errors and their recognition and the *t* test for comparing methods. The more explicit equation for the line (and correlation coefficient) although referred to, is judged too complex for presentation in this context.

Calculations used in radiology and nuclear medicine and some discussion of radioactive decay, the half-life and methods of calculation are the subject matter of another chapter. There is an appendix of tables and nomograms.

R. D. Allan.

**Manual of Clinical Immunology.** Edited by Noel R. Rose and Herman Friedman. Published by the American Society for Microbiology, Washington D.C., 1976. 932 pages, illustrated. Price $US16 Flexible binding, $US20 Cloth binding.

The preface states that this manual represents a joint effort by the American Society for Microbiology and the American Association of Immunologists to provide medical scientists and physicians interested in clinical immunology with a guide to the rapidly growing field of laboratory immunology.

It is directed mainly to laboratory personnel responsible for the performance of immunological tests in the clinical laboratory. The methods are described simply but with sufficient detail to avoid referral to other sources. The methods are those used by the authors and brief discussions of other methods are included.

The book contains 124 chapters grouped into the following sections. Tests for humoral components of the immunological response. Tests for cellular components of the immunological response. Immunoassays. Bacterial, mycotic, and parasitic immunology. Viral, rickettsial and chlamydial immunology. Immunohaematology. Laboratory examination of patients with allergic and immunodeficiency diseases. Autoimmune diseases. Tumour Immunology. Transplantation Immunology. Licensure and certification programmes in clinical immunology laboratories.

There are over 100 authors eminent in their field. They submitted to a peer group review procedure. The quality of the book reflects this painstaking process.

This is a very practical reference manual presenting basic information and not attempting to provide a compedium of all the available techniques and modifications. It was pleasing to find that the basic methods of immunodiffusion and immunoelectrophoresis still found favour with the authors and their techniques were all familiar. I found the book a mine of information on topics of personal interest. The assays for complement protein and the significance and interpretation of the results for example.

Curiously I could not find a reference to alpha$_1$ antitrypsin assay although there was a chapter on diseases of the lung. Two minor criticisms. Some pages were stuck together; possibly dampness in transit. The index was rather small for such a large book.

Sixteen dollars seems remarkably cheap for a work of this magnitude. R. D. Allan.

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# 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*: $m^2$, $cm^2$, $mm^2$, $\mu m^2$.
*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

When time permits, authors will be given the opportunity to correct proofs before publication but not to insert new material. Proofs must be returned within three days of receipt.

## Reprints

Reprints are available to authors at their own expense on a cost basis. Orders for reprints (minimum 50), must be given when returning the proofs.

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