MARCH, 1979

THE NEW ZEALAND JOURNAL OF

# medical laboratory technology

An Official Publication of the New Zealand Institute of Medical Laboratory Technology Incorporated



VOLUME 33, No. 1

# A Change in Style

This issue of the Journal marks an alteration in the size of the page and in the typeface used. This is the result of the introduction of an offset process which dispenses with the linotype operation and substitutes photosetting which is generally quicker. The copy is printed directly by a variable typeface machine and then photographed. Advertising material can be processed directly from suitable copy. This means repro proofs or bromides for single colour work and film positives for four colour work. Blocks no longer require to be made. One of the requirements of the process relates to the size of the paper used and this will result in a slightly smaller page. However, by using smaller margins the same amount of material can be printed.

The typefaces available are different from these previously used and I must admit to a twinge of regret at the passing of the stately, if unfashionable Bodoni typeface for headings. However, this technical advance should facilitate the production of the publications.

*Editor.*

# The New Zealand Journal of Medical Laboratory Technology

Volume 33, No. 1 March, 1979

The New Zealand Institute of Medical Laboratory Technology (Inc.)



The JOURNAL is published three times yearly (in March, July and November), and is distributed, without charge, to all financial members of the N.Z.I.M.L.T. (Inc.).

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# Pica

**Carolyn Reid**

**Haematology Department, Dunedin Hospital**
*From a Paper read to Conference 1978, Nelson*

**Summary**

Pica, a common symptom of iron deficiency, is the compulsive eating of substances, usually a single item of food, ice or dirt, something within easy reach of the victim. A clinical history, laboratory results and discussion presented concerning iron deficiency and Pica.

**History**

Master S—a seven-year-old child was an acute admission to Wakari Hospital on the 14 February 1978.

The patient had had vague epigastric pain for months and presented with severe colicky pain over the past three days. Accompanying the pain vague nausea had been present for months which developed into severe vomiting for the three days prior to admission. During this last period he would only tolerate a fluid diet.

On examination the patient had a very distended abdomen with what appeared to be an epigastric mass.

On enquiry the child proved to have an interesting history. He had been a slow eater, especially of meat for several years, and had a history of anaemia. In 1976 he showed marked iron deficiency—his haemoglobin was 62g/L and his MCH 16.3. No weight loss was recorded. He had a history of eating carpets and teddy bears and had been doing this for about two years (see figure 1).

A blood screen was performed and showed the following results.

The patient was grossly anaemic:

| | |
|---|---|
| Hb | 75 gm/litre |
| PCV | .27 |
| MCH | 17 pg |
| MCHC | 27 gm/litre |
| MCV | 63fi |

The blood film showed typical iron deficiency with marked hypochromia, bizarre shaped cells, microcytes, targetting and polychromasia.

He was transfused with one unit of packed cells which brought his haemoglobin to 120 g/litre and his film showed the resultant double population of patient's iron deficient cells and the normal transfused cells.

Figure 1.—If your teddy bear looks like this—see a doctor.

In view of the abdominal mass surgery was performed and two trichobezoars were removed (See figure 2).

As can be seen they were very large, were taking up the whole of his stomach space and had actually assumed the shape of his stomach. The maximum diameter was 15 cm.

Postoperatively the patient progressed well and was discharged with a haemoglobin of 112 gm on the 3rd March.

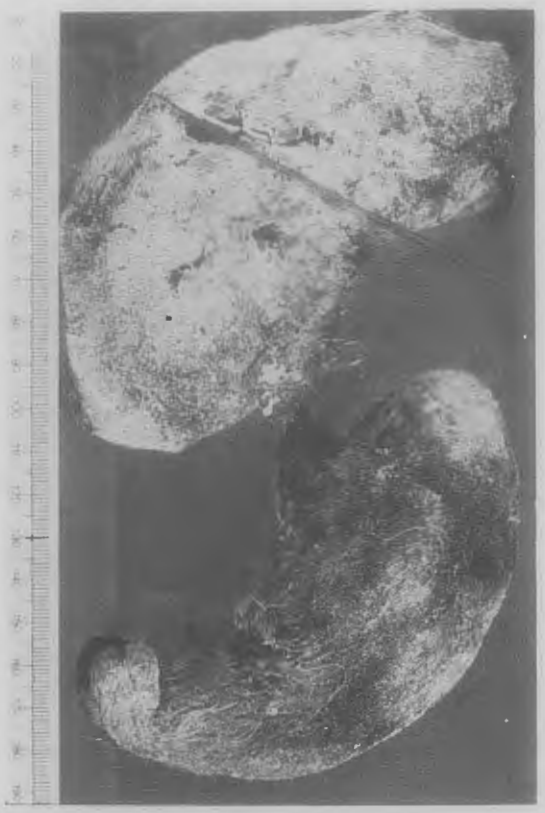

Figure 2.—Trichobezoars removed from the stomach.

**Discussion**

PICA (Latin, magpie) "A desire for strange foods, A craving to eat strange articles such as hair, dirt or sand."

Pica,[1,2,3] a common symptom of iron deficiency, is the compulsive eating of substances, usually a single item of food, ice or dirt, something within easy reach of the victim. Pica is not necessarily a disease of poverty or malnutrition or an emotional aberration, it is a symptom of iron deficiency, and is thought to occur in more than 50 percent of patients with iron deficiency anaemia.

Pica is not solely a disease of man however. Pica has been encountered by veterinarians in many animal species and is usually attributed to dietary deficiency rather than psychological factors.

Piglets are born with only very limited reserves of Fe and Cu because transfer of these elements across the placenta in sows is restricted. Hb levels in piglets decrease very rapidly especially in the first three days of life as the sow's milk is naturally low in Fe and Cu. During their first month, piglets kept in concrete pens will often avidly eat any lumps of earth or cut sods which are thrown into the pens. This apparent pica is considered by some investigators to supply other nutritional requirements, in addition to those for Cu and Fe. Piglet anaemia may be prevented in housed litters by simply providing an unrestricted supply of earth.

Until recently there has been much controversy—which comes first? Does iron deficiency cause pica or does pica lead to iron deficiency? Coltman[1] found in blind experiments that the iron deficient ice eaters lost the appetite for ice within two weeks after beginning iron therapy. The pica ceased before the anaemia was corrected.

When a woman eats laundry starch she is certainly in a negative iron balance and clay evidently contains substances which chelate ionized iron preventing its absorption. Ice however does not displace any dietary factor nor does water prevent the absorption of iron. It seems therefore that pica is a consequence not a cause of iron deficiency.

The degree of iron deficiency or anaemia need not be severe, the Hb level may be within the normal range and discovery of pica may be the first clue to the existence of iron deficiency.

The abnormal craving is not always for a strange food or article, the craving may be for something commonplace. The stories of pregnant women who send their husbands for strawberries or pickles at 2 a.m. could probably disappear from circulation if all pregnant women took supplementary iron. However, there are probably other reasons for such cravings.

The patient's local situation may influence the selection of the craving, for example, ice eating would pose a problem in communities with no electric refrigerators.

A sense of shame or guilt may interfere with eliciting the history of pica. The patient may consider his irrational behaviour ridiculous and disgusting.

It is not known how iron deficiency perverts the appetite. It is not compensatory since the materials eaten are not foods rich in iron. It is not

psychological because the craving disappears when the iron deficiency is treated, even when the doctor has been unaware of the existence of pica and the patient is unaware of the connection between the pica and the ferrous sulphate which is taken to correct the anaemia. Most of the reports concern women and children, but men have pica also.

It has been hypothesized that the control of appetite regulation is part iron dependent. It has been shown that cats can be induced to swallow inedible objects when certain points in the hypothalamic area are stimulated. Additionally nuclear masses in the same region differ from other brain centres in their high iron content. Thus iron depletion in the tissues around the appetite centre may result in pica.

Intensity of compulsive eating varies from one patient to another and the variety of foods eaten are quite remarkable. Some odd examples have been noted—one woman ate nine bunches of celery a day whilst another spent most of her time going backwards and forwards to the greengrocer to buy carrots, and her complexion was deep orange in colour from the ingestion of so many carrots. Yet another woman ate toilet paper. The foods selected are usually poor sources if iron and often are crunchy—peanut butter, clay, gum and paper being exceptions. Sad cases are those, for example, of ghetto children who eat peeling lead based paint and get lead-poisoning or matches and get phosphorus poisoning.

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## Correspondence

Sir,—I feel that I must make comment about the article by B. M. Lockwood on the Testing of Three Aminoglycosides which appeared in the July issue of the Journal (Lockwood, B.M., 1978).

I agree with his conclusion that the use of tobramycin warrants serious consideration. However, I am concerned about the method he has used to arrive at this conclusion.

Throughout the article he stresses the fact that tobramycin produced "larger zone sizes" than either gentamicin or amikacin, using 10 microgram discs for all three agents. Figure 1, labelled "Comparison of Aminoglycoside Sensitivities" shows a comparison on zone sizes produced to emphasize this point. Nowhere in this article does the author make any reference to M.I.C.'s. He only uses zone size cut off points to establish the sensitivity of the organisms. The basis of these reference figures is not clearly defined.

This method cannot be used as the basis for comparison of different agents.

The only reliable way to make a comparison between the in-vitro effectiveness of different antimicrobial agents, is by either broth or agar dilution methods (Ericsson H.M., 1971). To use agar diffusion methods without reference to some quantitative method ignores the fact that relative effectiveness is only one of the many factors which influence the production of a zone of inhibition. Other more important factors are diffusion rates, ionic interference, disc potency etc.

The sensitivity or resistance of an organism to an anti-microbial agent is the result of a combination of the intrinsic activity of the agent, and the tissue levels which can be achieved with reasonable safety in the patient. Both gentamicin and tobramycin are very similar, but not identical agents, in that the levels of activity weight for weight are almost comparable as are their pharmacological properties. However, it has been shown (Davis, 1975) that tobramycin is less affected by ionic interference than is gentamicin, and this alone could account for the smaller zones produced by the latter. Amikacin on the other hand is quite a different product. It is less active weight for weight than the previous two agents in many instances, but off setting this, it is tolerated to a much higher level in the serum and tissue. The accepted upper limit for gentamicin and tobramycin in serum is 10 micrograms per millilitre, whereas the figure for amikacin is 30

micrograms per millilitre. The use of a 10 microgram disc for amikacin in this instance, places this agent in a position of disadvantage. We have found that for routine testing of amikacin, the optimum disc content is 30 micrograms. The use of the lower potency disc may account for the alarmingly high figure of resistance (11 percent) found to this agent. With reference to cross resistance between gentamicin and amikacin.

Of the nine aminoglycoside inactivating enzymes exhibited by bacteria, amikacin is affected by only one, namely aminoglycoside-acetyltransferase-six-prime, whilst gentamicin is affected by six enzymes amongst which aminoglycoside-acetyltransferase-six-prime is only marginally active. The resistance exhibited by organisms to these two agents is independent.

In conclusion, all three of the above agents have a valuable place in the treatment of infections due to *Pseudomonas aeruginosa.* To promote any one of them on the basis of the results achieved in this study is misleading. Of interest is the fact that of a recent investigation into 28 isolates of gentamicin resistant *Pseudomonas aeruginosa* in this laboratory, only one was sensitive to tobramycin by M.I.C. Tests.

GRAHAM CAMERON,
Charge Technologist.
Microbiology,
Auckland Hospital.

8 September 1978.

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## Obituary

### Ivor Wendell Saunders

The death of Ivor Saunders is noted with regret. He died in Hospital on 29 November 1978. He was Principal Technologist at Taranaki Base Hospital until his retirement in 1961.

Ivor Saunders was one of the Foundation Members of the Institute and worked for 26 years in the laboratory at the New Plymouth Public Hospital. He began studying for a B.Sc. at Victoria University and completed his studies in the early 1920s at Auckland while working at the bacteriological laboratory. He then worked as a bacteriologist at Invercargill, later transferred to Napier and finally to Taranaki in 1935. At one stage he contracted tuberculosis through contact with specimens and had to submit to surgery. After his retirement he visited Europe for two years and returned to live in New Plymouth.

He leaves his wife, three sons and 10 grandchildren. Ivor Saunders will be remembered as one of the "Old Brigade," who struggled against considerable odds to establish the professional organisation which now flourishes. We should be grateful to them. **Editor.**

# An ABO Blood Grouping Anomaly

J. C. Coplestone

**Taranaki Base Hospital, New Plymouth**

*Student Essay Award, Technical Section, 1978*

**Summary**

Chronic Lymphatic Leukaemia has often been shown to be associated with low serum IgM levels and ABO blood grouping anomalies. This paper presents an example of this.

**Case History**

Mr RB, an 84-year-old male with late onset diet controlled diabetes, was admitted to Taranaki Base Hospital in August 1976 for a total hip replacement necessitated by osteoarthritis. During routine grouping he was shown to be group A positive but it was noted that his serum reaction with group B cells was weaker than normal when compared with other patients and donors sera tested with the same B cells. He received two units of $A_1$ positive whole blood during surgery and made an uneventful recovery.

He was readmitted 15 months later for investigation of weight loss and the following haematological values were found: ESR 42mm/h, Hb 114g/l, PCV 0.34, MCHC 335g/l, RBC 3.58 x $10^{12}$/l, MCV 94fl, WBC 57.0 x $10^9$/l neutrophils 11 percent lymphocytes 81 percent, monocytes 8 percent.

A diagnosis of chronic lymphatic leukaemia was made and he was started on a course of cobalt therapy.

Three months after this admission he was transfused three units of $A_1$ positive packed cells to correct his falling haemoglobin of 93g/l and at this stage his white count had dropped to 6.0 x $10^9$/l with 46 percent lymphocytes. Once again during routine grouping an anomaly was found, this time though his expected anti B was not able to be detected. Transfusion of donor blood commenced before further studies could be carried out.

Six weeks later his haemoglobin had dropped to 87g/l and another cross-match requested. This time adequate serum was available for further investigations. The lack of anti B in his serum was confirmed at 4°C, room temperature, and 37°C.

The patient's condition deteriorated and he died in April 1978, 20 months after his blood group abnormality was first noticed.

**Discussion**

A number of factors may influence the expected results between cell and serum ABO reactions. Mr RB's cells grouped as A, while his serum failed to react with A and B standard cells, indicating serum group AB.

There are many accounts of this sort of occurance in the literature. It could possibly be explained in several ways.

1. Depressed antibody production due to age.[2,3]
2. Excessive ABH substance in the patient's serum.
3. Production of IgG antibodies instead of IgM due to old age.[2]
4. Decreased A antigen titre, causing decreased production of anti B.[6]
5. Hypogammaglobulinaemia.[4,5,7]

As Mr RB was grouped previously as a group A with a low titre anti B, congenital absence of anti B was ruled out, as was the chance that he had a weak sub-group of B or that he was an AB with a B antigen modified due to his leukaemia.[1]

Mr RB's serum was reacted against a number of washed group B cells at 4°C, room temperature and 37°C, using group O cells in parallel. Issit and Issit[3] have shown that ABO agglutinins decrease in strength as individuals grow older, thus in elderly patients the agglutinins may be difficult to detect, and tests at 4°C may be necessary to demonstrate their presence. Mr RB's serum was tested in this manner and no reactions were observed.

No agglutination or haemolysis was observed when his serum was incubated with B cells at 37°C, although a very weak reaction was observed when monospecific anti human IgG was added, indicating insignificant, probably normal amounts of IgG anti-bodies present. Routine performance of ABO groupings using washed cells

ruled out the possibility of excess ABH substance present in his serum which could neutralise anti B. Titres showed his A antigen strength to be normal when compared to a panel of normal donor cells of group $A_1$ and $A_2$.

The remaining possibility was the lack of anti B due to hypogammaglobulinaemia. Protein electrophoresis and immuno-globulin levels were performed on his serum. See Table 1.

On the electrophoresis pattern the gamma region showed a small discrete band. A monoclonal gammopathy was indicated by immuno-electrophoresis which showed an abnormal IgG which was mirrored by the kappa light chain arc. Albumin was low, other fractions appeared normal.

As can be seen from the Table no IgM was detected and his IgA level was lower than normal. Mr RB's serum was concentrated five times using an Amicon Minicon concentrator with 25,000 mw cut-off, and his serum ABO grouping and immunoglobulins were repeated. His serum now showed weak reactions when incubated with a panel of B cells and no reaction with 0 cells at 4°C. His immunoglobulins showed a trace of IgM present (less than 0.1 g/l).

Latiner[5] states that there are two broad types of secondary hypogammaglobulinaemia. In one type there is a tendancy for the suppression first of IgM, then IgA and finally IgG. Depending on the cause, the sequence may take months, (toxic) or years, (lymphoid hyperplasia). This type of hypogammaglobulinaemia can be due to circulating factors suppressing immunoglobulin synthesis. This situation can occur in relation to prolonged uraemia, severe infection, cytotoxic therapy etc. It is found in an appreciable proportion of patients suffering from the various types of lymphoid hyperplasia (Hodgkin's disease, chronic lymphatic leukaemia etc).

**Table 1**

| | | |
|---|---|---|
| Total Protein | 53g/l (Normal 60-80) | |
| Albumin | 21g/l (Normal 29-40) | 39% (Normal 50-70) |
| $\alpha_1$ globulin | 4g/l (Normal 2-5) | 7% (Normal 2-6) |
| $\alpha^2$ globulin | 9g/l (Normal 7-11) | 17% (Normal 5-11) |
| $\beta$ globulin | 3g/l (Normal 8-12) | 6% (Normal 7-16) |
| $\alpha$ globulin | 17g/l (Normal 7-16) | 31% (Normal 11-22) |
| IgG | 10.2g/l (Normal 5.0-16.0) | |
| IgA | 0.86g/l (Normal 1.2-4.2) | |
| IgM | none detected (Normal 0.5-1.7) | |

Teitelbaum *et al.*[3] confirmed this by showing a general tendency toward low titres of IgM anti A and anti B in patients with chronic lymphatic leukaemia when compared to a normal population.

Suppression of immunoglobulin in chronic lymphatic leukaemia (or other lymphoid disorders) can manifest in many ways including loss of immunity in the form of blood group antibodies. There may be several reasons for this.

**Acknowledgments**

My thanks to Mr M. R. Butler for the biochemical results and Dr A. E. White and Mr R. J. Austin for advice and assistance in the preparation of this paper.

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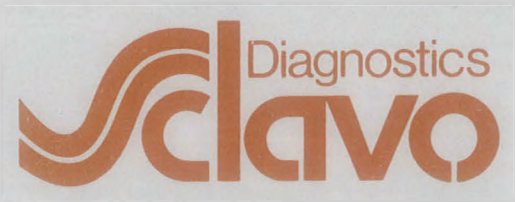


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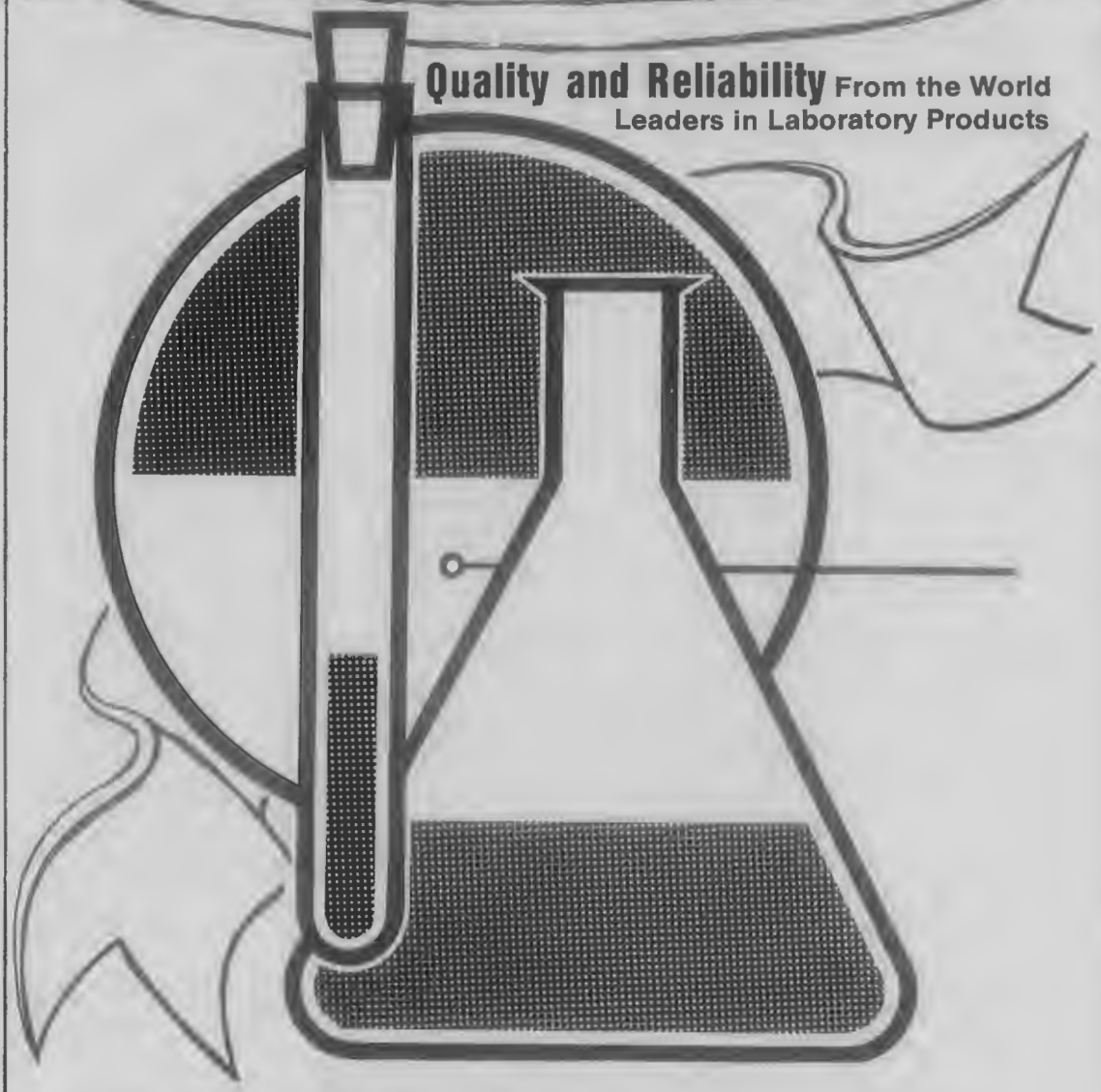

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# A Case of IgD Myeloma

**Susan Walker**

Chemical Pathology Division, Department of Laboratory Services
Wellington Hospital
*Received for Publication, July 1978*

## Introduction

IgD immunoglobulin was first identified by Rowe and Fahey in 1965. Rowe DS *et al.* (1965)[6]. IgD constitutes less than 1 percent of the total serum immunoglobulins and IgD myelomas are quite rare. To date the existence of only a few cases with this type of paraprotein have been documented in the medical literature. Consequently it appears worthwhile to report an additional case of an IgD myeloma which exhibited unusual features: kappa light chains in the IgD paraprotein and the presence of two paraproteins in the serum.

## Clinical Features

A 62-year-old female was admitted with a three-month history of malaise and a three-week history of severe thoraco-lumbar back pain. She had suffered a collapsed vertebra in 1975. Bone X-rays and a bone scan showed osteolytic lesions in the skull and femurs but none were seen in the spinal column.

## Laboratory Investigations

On admission the sedimentation rate was 95 mm/hr, haemoglobin 100 g/l, haematocrit 0.278, urea 12.9 mmol/l, creatinine 0.19 mmol/l, potassium 4.0 mmol/l, calcium 3.08 mmol/l, phosphate 1.20 mmol/l, total protein 84 g/l and albumin 41.2 g/l. Bone marrow aspiration showed a 60 percent plasma cells, plasma blasts or plasmacytoid lymphocytes. Cellulose acetate electrophoresis of the serum showed a discrete band at the end of the gamma region. The gamma globulin was 19.0 g/l, Agarose gel electrophoresis showed two distinct bands in the gamma region.

Serum immunoelectophoresis showed a short discrete arc reacting with IgD antisera, the free and immunoglobulin bound kappa and free kappa antisera. A discrete arc corresponding in position to that of the IgD arc was also seen with the whole human antiserum. All antisera were from Oxford except for the anti-IgD which was from Behringwerke.

Immunoglobulin measurements by radial immunodiffusion Mancini *et al.* (1965)[3] using Behringwerke Tri-Patrigen plates showed IgG 5.2 g/l (normal range 6.6-14.0 g/l), IgA less than 0.20 g/l (normal range 0.8-3.8g/l) and IgM less than 0.20g/l (normal range 0.6-2.8g/l). The amount of IgD present was quantitated using a Behringwerke LC IgD partigen plate with a 1:50 dilution of the patient's serum with saline and was found to be 15.5 g/l (average value 0.30 g/l).

Total urine protein was 10.1 g/l and after five-fold concentration, cellulose acetate electrophoresis revealed a very small amount of albumin and a large discrete band in the gamma region. Urine immuno-electrophoresis showed a short discrete arc that reacted with free and immunoglobulin-bound kappa, free kappa (Bence Jones) but not with IgD antisera.

## Discussion

The frequency of IgD paraprotein in a combined series was 1.3 percent Colls, B.M. *et al.* (1975)[1]. Nearly all IgD paraproteins have been of lambda type and this is in keeping with the 87 percent incidence of lambda type in normal plasma cells which contain IgD Pernis, B. *et al.* (1969).[5] In one study Hobbs, J.R. (1975)[2] 37 of 38 patients with IgD paraprotein were found to have lambda light chains. Our case is unusual because the patient had not only an IgD kappa paraprotein in the serum but also a second band which was shown to be a Bence Jones kappa paraprotein. The incidence of multiple paraprotein bands in serum electropherograms is low. Of 6141 recorded cases of myeloma, only 60 (1 percent) had more than one paraprotein band Oriol R *et al.* (1974)[4]. The question arises whether the cells producing the abnormal globulins belong to the same or different clones. In our patient this question cannot be answered since it is not

known whether the free kappa chains in the serum are different from those associated with the IgD paraprotein. Unequal production of light and heavy chains may occur in patients with multiple myeloma. The concentration of Bence Jones protein in the serum depends on its rate of synthesis, excretion and catabolism Wochner, R.D. *et al.* (1967).[7] In persons with normal kidneys, about 90 percent of the glomerular-filtered light chains are reabsorbed and catabolized by the renal tubules. In renal insufficiency the light chains are retained in the serum and their biological half life is greatly increased. In our case, the second paraprotein band in the serum, i.e., the Bence Jones kappa, could have been due to the retention of light chains because of the patient's renal insufficiency, but this is unlikely since renal function was minimally depressed.


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# A Solid State Relay for use in Technicon or other Fixed Temperature Heating Module


**John D. Newton, ANZIMLT,**

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*Received for Publication, July 1978*

## Introduction

The fixed temperature heating modules of Technicon Auto Analysers, such as oilbaths and dialyser baths, have the power to the heating elements switched on and off by a relay controlled by a contact thermometer. In the generation I modules this relay is a sealed mechanical device which after prolonged use may jam and cause potentially dangerous overheating. In the generation II modules this relay is a solid state printed circuit board, part number 157-B277-01. This printed circuit although not prone to jamming, can be destroyed spectacularly in the event of chemical leakage into the temperature controller housing at the bottom of the heating bath.

This paper is in two parts, the first shows how the solid state relay can be replaced at a saving of not less than $100. The second part describes how the basic circuit for the solid state relay can be utilised as a direct replacement for the mechanical generation relay, part number 660-5000-01, this latter at a saving of about $40. Other uses for the circuit may suggest themselves to readers, for example, as the basis for fixed temperature heating blocks or waterbaths.

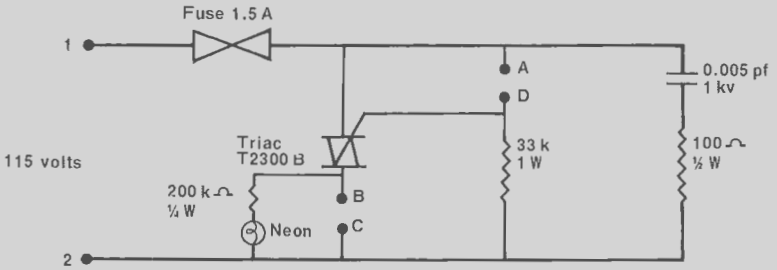
## Part I

The printed circuit board, 157-B277-01, is a solid state relay module which turns the power on and off to the heating element in the generation II, and SMA fixed temperature heating baths.

### Diagram 1

It comprises a triac connected in series with the heating element, a fuse and the 115 volt power supply. The triac is gated by current, from the 115 volt supply, limited by a 33 k ohm resistor. The contact thermometer is wired so that when it is closed, that is when the set temperature is reached, the power to the gate of the triac is short circuited and the triac then becomes non-conducting. The heater, in series with the triac, thus becomes turned off. A neon indicator light is connected in parallel across the heater as a visible sign that the heater is on. Current to the neon light is limited by a 200 k ohm resistor. A 100 ohm resistor and a 0.005 picofarad capacitor in

Diagram 1




Connections to Circuit Board 157-B277-01

1,2 115 volt power supply
B,C Heating element
A,D Contact thermometer

series across the 115 volt supply helps damp out current "spikes".

The board 157-B277-01, cost $110.10 in February 1978. The same circuit can be assembled to do the same job for $8.13. Components required are as follows, prices as at February 1978.

| | | |
|---|---|---|
| "Marking", 157-0447-01 (Technicon) (This is the board without components on which 157-B277-01 is built) | one | $3.50 |
| Triac, RCA T2300B (or T2300D) | one | $3.20 |
| Fuse clips | two | $0.16 |
| Fuse, 1.5 Amp slo-blo | one | $0.20 |
| Capacitor, ceramic 0.005 picofarad, 1 k volt | one | $0.35 |
| Resistor, carbon 100 ohm, ½ watt | one | $0.06 |
| Resistor, carbon 33 k ohm 1 watt | one | $0.10 |
| Heat sink, SF2 for Triac | one | $0.25 |
| Neon lamp, 80-100 volts | one | $0.25 |
| Resistor, carbon 200 k ohm ¼ watt | one | $0.06 |
| | Total | $8.13 |

The triac was purchased from AWA Wellington, and the other components from the Wiseman Electric Company Wellington, except for the bare board 157-0447-01, which was purchased from Technicon. As can be seen, the cost of the components compares very favourably with the cost of the complete board.

The entire job, removal of the heating bath, removal of the circuit board, reconstruction of the circuit board, replacement of the board and replacement of the heating bath in the analyser can be accomplished within one to two hours depending on experience.

**Part II**

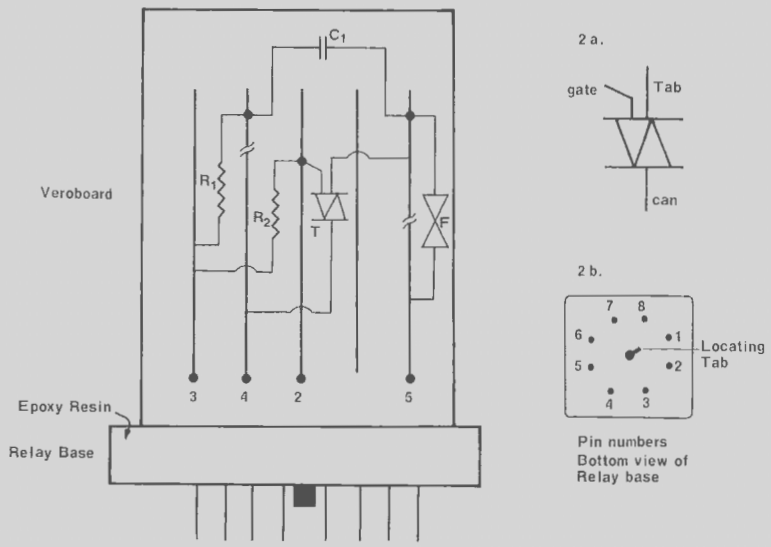
The generation 1 heating baths and dialyser baths rely on a contact thermometer to operate a mechanical relay (660-5000-01) to control power to the heating element. Once one of these relays has jammed and been freed it is forever suspect and the safest recourse is to discard it and replace it with a new one. The current cost is close to $50.

It is, however, possible to use the base of the faulty relay and build a solid state relay based on the circuit in part one above. This solid state relay is completely interchangeable with the mechanical one and requires no modification of the heating bath itself.

To make such a relay, take a mechanical relay and with a hacksaw carefully cut away the hermetically sealed can from the base. With care the relay itself can then be removed leaving the connecting pins in the relay base intact, some brute force carefully applied via a pair of stout wirecutters is satisfactory.

A piece of "veroboard", available in sheets or as offcuts from any electronics supplier, is cut ap-

Diagram 2




$C_1$ – Capacitor 0.005 picofarad, 1 k volt
$R_1$ – Resistor 100 ohm, ½ watt
$R_2$ – Resistor 33 k ohm, 1 watt
T – Triac RCA T2300 B, or T2300 D
F – Fuse Slo-blo 1.5 Amp
2.3.4.5 – Connections to numbered pins on relay base
≈ – Breaks in copper strips of veroboard

proximately 3 cm x 5.5 cm with the copper strips lying along the length of the veroboard. Trim about 5 mm of the copper strips from both ends of each strip, this is to reduce the risk of accidental short circuit or electrocution. Using a rapid setting epoxy resin, such as Rapid Araldite, or Bostik Quickset Epoxy, glue the veroboard into the old relay base so that it is standing on end diagonally across the base. Once the resin has set the components can be soldered on. It is easier if the relay can be supported in its proper socket i.e., on top of a heating bath.

**Diagram 2**

Diagram 2 shows the approximate physical layout of the components. The components required are the same as those in Diagram 1, with the exception of the resistor 200 k ohm and the neon lamp. These components already exist as part of the wiring to the relay socket on the heating bath. The connections 3, 4, 2 and 5 on Diagram 2 are soldered to the same numbered pins on the relay base, short pieces of insulated wire are used. Diagram 2b illustrates the numbering of the pins on the relay base. Diagram 2a illustrates the connections to the triac. If the tab and can connections are reversed, the triac does not turn off, and the heater stays on.

Underneath the relay socket on the heating bath the connections are as follows; pin 5 is common to 115 volt power and the contact thermometer, pin 2 is the second connection to the contact thermometer, pin 3 and 4 are the heater connections with the neon indicator connected in parallel across them. Pin 2 is also the second 115 volt power connection.

The triac RCA T2300B is the same as the triac fitted to the Technicon board 157-B277-01, it is a 200 volt 2.5 amp triac and is satisfactory for 115 volt applications. The T2300D is a 400 volt 2.5 amp triac and gives a little more safety margin against voltage "spikes", it should also be adequate for 230 volt applications, and should cope with heaters of up to about 500 watts.

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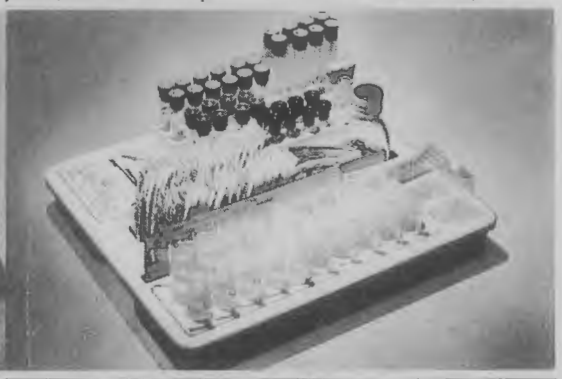

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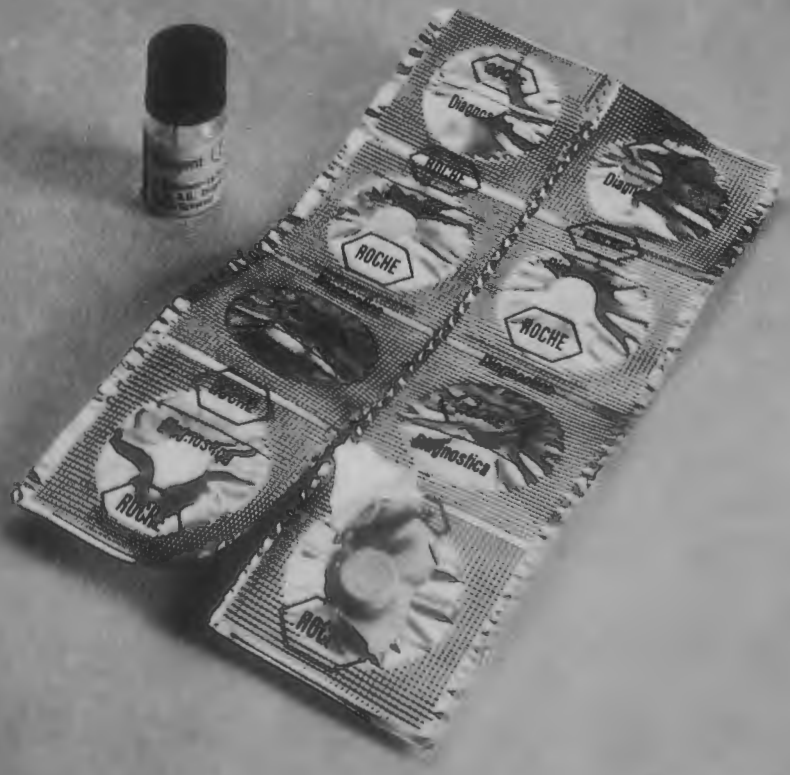


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**Evaluation**

A reconstructed solid state relay, equivalent to part 157-B277-01, has been in use in our SMA 6/60 since February 1978 without failure. Two solid state relays, direct replacement for part 660-5000-01, are currently in use in 95°C heating baths, one has been in use since February 1978, the other was installed in June this year. Both were installed in response to failure of the mechanical relays, and so far neither has failed. An additional bonus from their use is that they are much more sensitive to control by the contact thermometer and there is not the usual degree of temperature variation in the heating bath as the heating element cycles on and off.

**Conclusion**

Two Technicon parts, 157-B277-01, and 660-5000-01 can be replaced at a cash saving of $101.97 for the first part, and $44 for the second part. In the case of the second part a greater degree of reliability is anticipated. Consideration is also being given to the possibility of making aluminium incubation blocks using this circuit.

*Readers intending to modify equipment should use the services of qualified personnel and take note of the N.Z. Electrical Safety Regulations.—Editor.*

# Oxygen Electrode Calibration A Simple Inexpensive Method

**M. Legge**

**Perinatal Biochemistry Unit, Pathology Services**
**Christchurch Women's Hospital**
*Received for Publication, July 1978*

**Introduction**

The calibration or assessment of quality control of the Clark oxygen electrode is, in general, dependent on using known concentrations of oxygen, commercial quality control products, tonometered blood or air equilibrated water. These methods will give one or two check points but are strictly limited in their usefulness. Alternative methods for assessing the state of the Clark polarographic oxygen electrode have been, the use of oxygen consuming reactions using NADH and sub-mitochondrial particles,[3] oxidation of NADH by phenazinium methylsulphate[2] and the consumption of oxygen by xanthine in the presence of xanthine oxidase.[1]

An oxygen consuming reaction has been described which is quantitative, inexpensive and does not utilise enzymes.[2,5,4,6] In this paper the technique has been investigated for its suitability to assess blood gas oxygen electrodes.

**Materials and Methods**

Phenylhydrazine hydrochloride and potassium ferricyanide were both obtained from Sigma Chemical Company, St Louis, USA. A 10mM phenylhydrazine hydrochloride solution is prepared in distilled water, this is stable for several hours at +4°C. Potassium ferricyanide, 1.0 mM, is prepared in a 0.05 M potassium phosphate buffer pH7.8. This reagent is stable for weeks at room temperature.

*Blood Gas Oxygen Calibration*

To check the calibration of the $PO_2$ electrode a Corning EEL 165 Blood Gas Analyser was used. The $PO_2$ electrode was first calibrated according to the manufacturers instructions. To a 5 ml syringe, 1 ml of potassium ferricyanide was pipetted. (For simplicity and speed it is better to withdraw the plunger and block the exit luer of the syringe with a finger.) A known volume (2-40 $\mu$l) of the phenylhydrazine hydrochloride is then added to the potassium ferricyanide in the syringe using a micro syringe or micro pipette. The reaction mixture is then mixed by agitation and injected into the sample chamber of the Blood Gas Analyser.

As the reaction is dependent upon the concentration of phenylhydrazine added to the ferricyanide solution, once the phenylhydrazine

has been consumed the residual oxygen tension in the sample remained constant for at least one hour when not exposed to the air.

To assess the suitability of the technique, seven different micro-volumes of phenylhydrazine hydrochloride were used to give differing oxygen tensions.

**Results**

The results for quintiplicate assays using phenylhydrazine hydrochloride solution volumes between 2 $\mu$l and 40 $\mu$l performed within the same day are shown in Table 1.

Table 1

| $PO_2$ MM Mercury Phenylhydrazine Hydrochloride Volume $\mu$l | n | x | SD | x + 2 SD | x — 2 SD |
|---|---|---|---|---|---|
| 2 | 5 | 151 | 1.1 | 153 | 149 |
| 6 | 5 | 138 | 1.3 | 140 | 135 |
| 10 | 5 | 125 | 2.1 | 129 | 121 |
| 16 | 5 | 105 | 1.6 | 108 | 102 |
| 20 | 5 | 91 | 1.5 | 94 | 88 |
| 30 | 5 | 57 | 1.9 | 61 | 53 |
| 40 | 5 | 18 | 2.1 | 22 | 14 |

Table 1.—Oxygen consumption by increasing the concentration of phenylhydrazine during an eight-hour period. n = number of observations, x= mean of the observations, SD = standard deviation.

Table II

| $PO_2$ MM Mercury Phenylhydrazine Hydrochloride Volume $\mu$l | n | x | SD | x + 2 SD | x — 2 SD |
|---|---|---|---|---|---|
| 2 | 30 | 154 | 3.9 | 162 | 146 |
| 6 | 30 | 139 | 1.7 | 142 | 136 |
| 10 | 30 | 126 | 2.7 | 131 | 121 |
| 16 | 30 | 105 | 2.1 | 109 | 101 |
| 20 | 30 | 92 | 2.2 | 96 | 88 |
| 30 | 30 | 57 | 3.7 | 64 | 50 |
| 40 | 30 | 14 | 3.9 | 22 | 6 |

Table II.—Oxygen consumption by increasing the concentration of phenylhydrazine during a 30-day period. n = number of observations, x= mean of the observations, SD = standard deviation.

The results from assays performed over a period of 30 days using the procedure as described are shown in Table II.

**Discussion**

The method using phenylhydrazine hydrochloride and potassium ferricyanide for oxygen electrode assessment is rapid, precise, convenient and cheap. Although the overall mechanism of the reaction is not known the probable sequence of events is that phenylhydrazine is oxidised to phenyldiimide by ferricyanide. The phenyldiimide then reacts very rapidly with oxygen, probably by a free radical reaction.[2-1.4]

This technique offers the possibility of comparing oxygen tensions on Clark polarographic oxygen electrodes from different blood gas equipment. The phenylhydrazine hydrochloride solution is stable for several hours at 4°C and can therefore, be transported without the problems associated with changes in oxygen tensions during transport. The method also offers as many check points as desired simply by using a different volume of phenylhydrazine hydrochloride to provide an oxygen tension. In the authors laboratory the method has been used to monitor the performance of oxygen electrodes after initial installation and has detected deterioration of the electrodes performance when the response falls outside defined limits. The use of pH 7.8 buffer also offers a pH control, although it cannot be made more acid.

As the method can be used on any Clark polarographic electrode it has also been used to monitor the performance of the Beckman glucose analyser electrode in the author's laboratory.

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# Comparison of Laboratory Performance in Two Interlaboratory Surveys: Digoxin, a Case Study

T. A. Walmsley, M. Lever, D. J. Munster, R. T. Fowler, and G. Tisch

Clinical Biochemistry Department, Christchurch Hospital
*Received for Publication, August 1978*

**Summary**

For the same six-month periods the same laboratory was ranked by the end-of-period report of the Wellcome Survey to perform the best digoxin assays in New Zealand whereas the MRC-funded Experimental Survey classified it as performing unsatisfactory digoxin assays, with a ranking four out of eight for precision. The results obtained on the Wellcome Survey specimens showed a high standard of reproducibility of results on replicate specimens, but examination showed that this was without consistency between these reproducible results and digoxin concentration. The apparent conflict in survey assessments is resolved if the analytical errors include non-specificity and non-linearity, which are included in the consistency or "precision" statistic of the experimental survey, whereas the Wellcome ranking is based only on reproducibility. The need to be alert to the meaning and application of survey statistics is highlighted.

**Introduction**

This laboratory participates in both the Wellcome Group Quality Control Programme‡ and the MRC-Funded Experimental Survey.* The end-of-period Wellcome report for the period 11 April 1977 to 12 September 1977 rated the performance as 12 out of 182 laboratories in the world and first out of seven laboratories in New Zealand. The MRC-Funded Survey for the same period (April 1977 to September 1977) classified the performance as unsatisfactory according to the survey criteria, ranking it fourth out of eight laboratories for precision. What do all the statistics mean, in terms of ability to measure digoxin and how can one survey rate performance as first in seven New Zealand laboratories, whilst the other rates the same laboratory over the same period as fourth out of eight. In order to answer these questions, the basis on which each survey is designed must be studied.

**Methods**

In the Wellcome Survey a total of 12 specimens of non-human based sera are analysed at fortnightly intervals. The 12 specimens in the survey are prepared from four pools of bulk sera; usually three specimens are prepared from each of the four pools by a variable fill procedure. The results obtained are compared with the mean of all laboratories using the same method code, the difference being expressed in terms of "standard deviations from the mean." Changes in these standard deviations throughout the survey period are used by laboratory staff as an assessment of accuracy and precision; even though this simplistic approach has been shown to be sometimes misleading[1]. At the end of the survey period, corrected results within each of the four pools are calculated using the dilution factors used to prepare each of the specimens in the survey. From this information, accuracy (mean bias) is calculated as the average difference between the laboratory mean obtained on each pool and the all-method mean for each pool. Precision is calculated as the average precision (standard deviation) achieved about the mean obtained on each of the four pools. The performance of the laboratory is assessed by ranking the precision achieved by the laboratory.

In the Experimental Survey a total of six pairs of human sera are analysed at monthly intervals. Each monthly report consists of a Youden diagram which displays results from all laboratories and the "target values." From the Youden diagram it can be seen where a laboratory's pair of results lie in relation to the target values and the results from other laboratories. No statistics such as the interlaboratory standard deviations are given. The target values for digoxin are derived from known

‡Wellcome Reagents Limited, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, United Kingdom.
*Survey organiser: Dr M. Lever, Biochemistry Department, Christchurch Hospital.

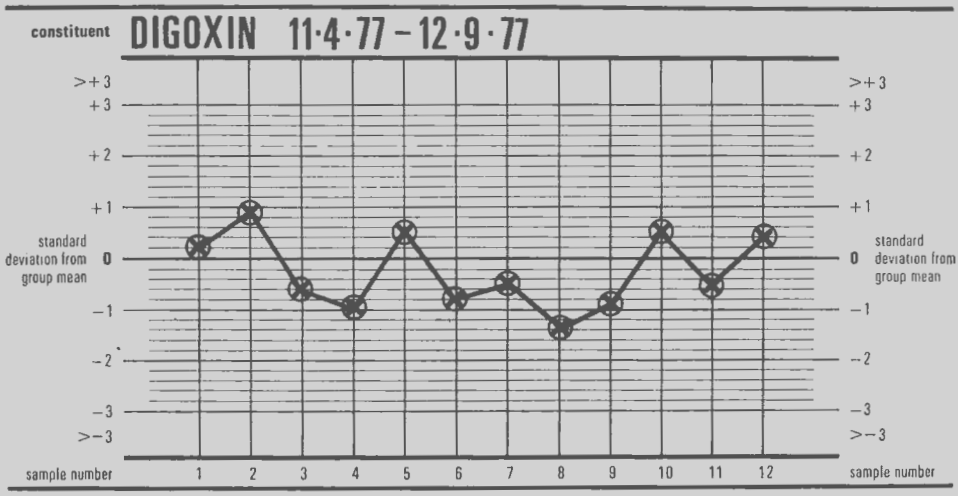


Figure 1.—Plot of the difference between the laboratory result and the method mean expressed in interlaboratory standard deviations from the method mean.

quantities of digoxin added to digoxin-free sera. At the end of the survey period the 12 laboratory results are plotted against the target values and the results analysed by linear regression. A visual interpretation of the graph can be used to assess the accuracy and precision of the method. The slope and intercept of the line of best fit and the coefficient of non-determination (CND) are also given. The CND is used as a measure of imprecision and reflects the degree of scatter of the points about the regression line. The laboratory's performance is assessed by ranking the CND achieved by the laboratory.

**Results**

During the Wellcome Survey period a plot was made of the standard deviations that each result was away from the mean (Fig. 1). From an ongoing assessment of Fig. 1 it would appear that the precision of the method was poor and that the method may be biased low. However, from the end-of-survey report the precision of the method was assessed as good, contradicting therefore the visual interpretation of Fig. 1. The end-of-period assessment of precision gave a standard deviation of 0.13 nmol/l with a bias of —0.46 nmol/l.

The Wellcome Survey results were analysed by the linear regression technique used in the MRC Survey; results reported to Wellcome (y axis) were plotted against the method mean (x axis) (Fig. 2). In the regression analysis the line of best fit through the points had a slope of 0.48 and an intercept of +1.00 nmol/l. The square of the correlation coefficient ($r^2$) was found to be 0.43 which gives a CND of 0.57 (since CND = $1-r^2$). This low slope and positive intercept agreed with the MRC Survey report (slope 0.71, intercept +0.28 nmol/l). To compare the precision in each survey it is necessary to take account of the total variance of the specimens used in the calculation of the CND. The CND is related to the total variance by the following equation:

$$\text{CND} = \frac{(\text{Syx})^2}{(\text{Sy})^2} \qquad \text{Equation 1}$$

where Syx = standard error of y upon x, this is equal to laboratory standard deviation if (a) non-specificity is insignificant compared with imprecision, (b) gross non-linearity is absent, and (c) imprecision is not strongly concentration dependent.

$(\text{Syx})^2$ = unexplained variance.

Sy = standard deviation of all laboratory's results

$(\text{Sy})^2$ = total variance of all the laboratory's results

CND = coefficient of non-determination or proportion of total variance that is not explained by regression—a measure of the scatter about the regression line.

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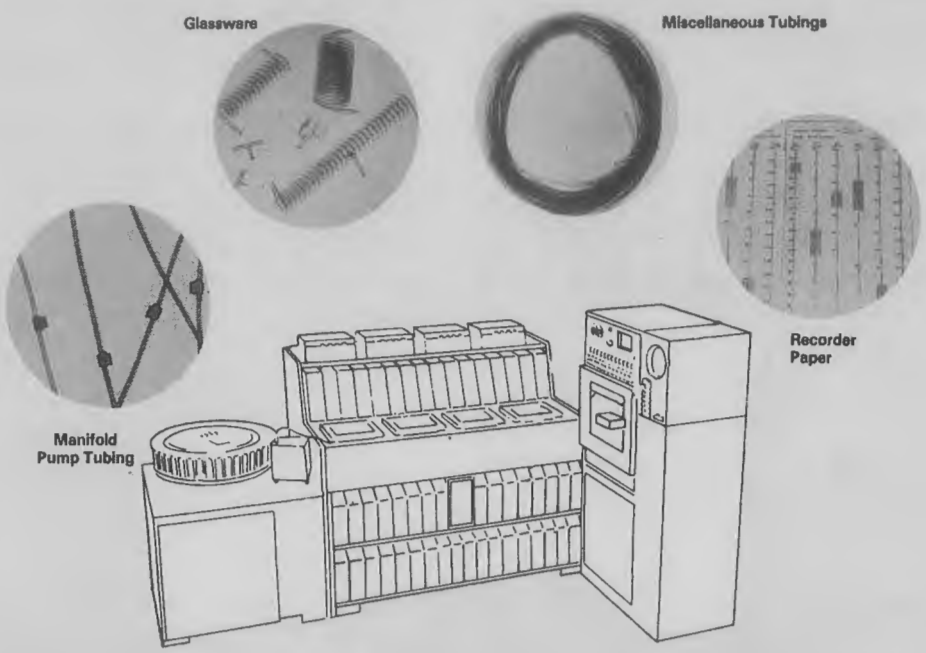


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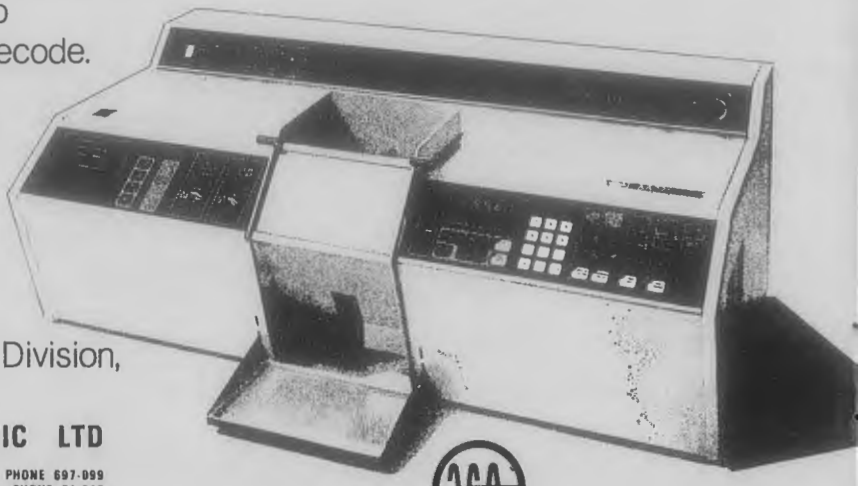

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Substitution of the total variance of the survey results in Equation 1 gave a standard deviation of 0.29 nmol/l for a CND of 0.57 from the Wellcome Survey data and a standard deviation of 0.22 nmol/l for a CND of 0.061 from the MRC experimental survey data. Therefore the apparent "imprecision" of the method, quantitated as Syx from linear regression of the Wellcome results is more than double the standard deviation calculated by Wellcome (0.13 nmol/l). The comparable figure in the experimental survey is similar (though smaller than) Syx calculated from Wellcome data. The reason why the CND is much larger in the Wellcome Survey even though the precision estimates are comparable is that the range of values in the survey is only from 1.5 to 2.6 nmol/l compared with the experimental survey range of 0.0 to 3.0 nmol/l.

Wellcome's assessment of precision is based on the ability to reproduce the four pool values in the Survey. The corrected value of each of the 12 specimens and its corresponding method mean was calculated from the dilution factors supplied by Wellcome. From the corrected results the average precision as estimated by Wellcome over each of the four pools was 0.13 nmol/l.

In Fig. 3 is a plot of the survey results corrected for dilution (y axis) against the method mean also corrected for dilution (x axis). From Fig. 3 it can be seen that this laboratory is indeed precise for each of the four pools and hence the reproducibility within pools is good but it is also evident that pool No. 2 in our laboratory compared to other laboratories using the same method, yields a much higher response. Hence the estimation of accuracy (bias) as used by Wellcome (which averages the difference between the mean of each pool and the all-method mean for each pool) will give a meaningless result in this case because of the differing responses of the pools in the assay. Referring back to Fig. 1, it can now be appreciated that the wide fluctuations in the "standard deviations from the mean" reflects a variable matrix effect rather than poor repeatability.

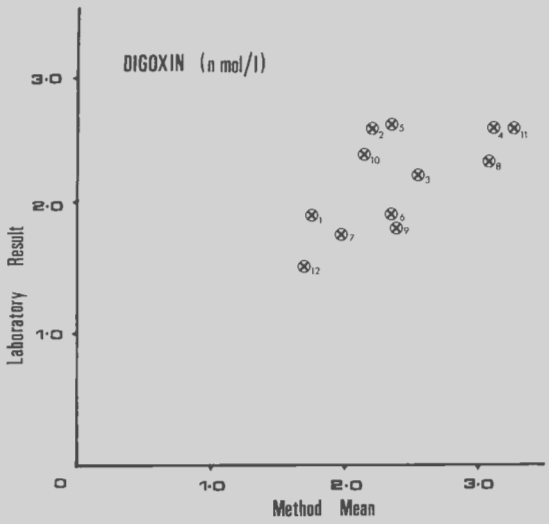


Figure 2.—Plot of laboratory result against the method mean. Each point in labelled with its corresponding sample number to allow easy cross-reference between Figs. 1, 2 and 3.

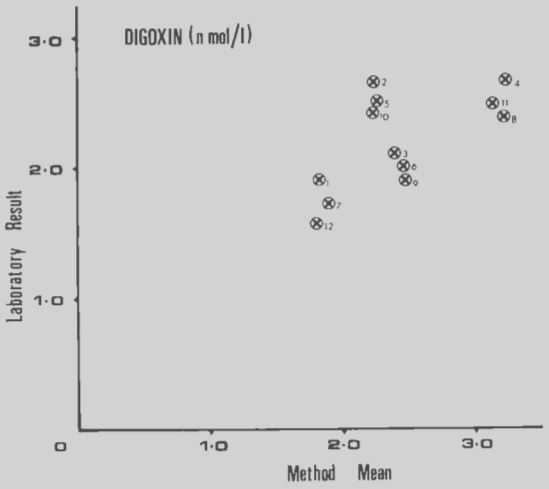


Figure 3.—Plot of laboratory result against the method mean after correction for dilution. Each point is labelled with its corresponding sample number to allow easy cross-reference between Figs. 1, 2 and 3.

**Discussion**

A laboratory participates in an interlaboratory survey for several reasons, the most important of which is to obtain an external audit of its ability to provide test results which meet clinical requirements[2]. When different surveys provide conflicting assessments of laboratory competence the laboratory is forced to investigate the exact nature of the surveys in order to find out exactly what each survey is assessing. Both of the surveys discussed have indicated inconsistency between reported results and the serum digoxin content,

but the summary Wellcome report appeared to show a high standard of performance.

Although this laboratory was alerted by MRC-Funded Experimental Survey results that its digoxin results were not satisfactory, as can be seen from the data here, the same conclusion could have been drawn from a close scrutiny of Wellcome survey results alone. The clues were there: there were large swings on the fortnightly charts (Fig. 1), and by drawing the regression diagrams shown in Fig. 3 (which were calculated from data routinely supplied by Wellcome), the existence of a problem became evident. This laboratory could replicate results for its digoxin assay much better than could most other laboratories in the world (Fig. 3) and this is what most workers consider to be good precision: however, the question of whether the assay is actually measuring digoxin is not tested in this evaluation of precision.

From our observations many laboratory directors base their assessment of laboratory performance on a quick review of end-of-period reports, and few laboratories have specialist staff with the time and experience to evaluate Wellcome survey data as described here. It is doubtful whether the problem outlined here would have been located had it not been for the conflicting survey reports. The end-of-period Wellcome report could have been misleading by giving a false impression that all was well, in which case there would have been no incentive to investigate further. *Even the briefest end-of-period report (and the briefest is the simple "rank" which is favoured by many pathologists as an encapsulated summary of their relative performance) should be based on an assessment of ability to meet clinical requirements.* The clinician does not merely require that, given the same specimen, the laboratory will always come up with the same result. The clinician wishes to know the status of a physiological variable (i.e., concentration of an analyte), and to do this he requires a "precise" laboratory to obtain the same result from *different* specimens having the same concentration of the analyte: *a fully consistent laboratory test will yield results in which the variation of test results is directly linearly related to the physiological variable.*

The MRC-Funded Experimental Survey is an attempt to design a survey protocol which meets these requirements. The case studied here illustrates the difference in approach. The "precision" statistic (CND) used in the experimental programme is usually closely related to imprecision but is also affected by other factors which occasionally affect the consistency of laboratory results; the two which are probably most important in the present case are serious non-specificity and marked non-linearity. The disadvantage of this approach is that the experimental survey results by themselves would have classified this laboratory as "rather imprecise" (i.e., inconsistent) without showing that the inconsistency derived from some factor other than inability to reproduce results: however, internal quality control data should be able to establish this reproducibility. Thus from this case study it is possible to derive some advice on the interpretation of data in the two surveys concerned:

1. In the Wellcome survey, one should plot one's own results against all-method means: *where the range of concentrations is sufficient,* this will often indicate inconsistency not otherwise noted. This procedure is especially important when the impression gained from a visual inspection of the on-going charts do not agree with end-of-period reports.

2. In the MRC-Funded Survey, if the precision ranking is poor whereas internal quality control indicates good precision, check whether the method is specific and linear. Another factor to check is the concentration-dependence of errors; that is, does the assay become unreliable at concentrations markedly different from your controls? These questions may need outside assistance to check, and one way to obtain this is to request a range of calibrated sera from survey organisers or other sources.

**Acknowledgments**

Financial support from the Medical Research Council is acknowledged. Constructive discussions with M. H. Abernethy and C. M. Andre, and their approval to publish, have made this report possible.

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# Non-Chemical Errors in Chemical Pathology—A Study

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*From a paper read to the NZIMLT Conference, Nelson, 1978.*

**Summary**

Chemical pathology departments spend thousands of dollars each year on quality control material in order to detect and avoid serious chemical errors. However, errors other than those of a chemical nature can and will occur, such as transcription, chart reading and calculation errors. Over a three-week period, a study was undertaken in the routine section of our chemical pathology department to detect these latter types of errors, and of nearly 25,000 tests performed, 117 such errors were detected (0.48 percent of total tests performed).

**Introduction**

In 1969 in Great Britain, McSwiney and Woodrow[1] performed a study of errors in their chemical pathology department and found the error rate to be 2.3 percent of all tests performed. Of these errors 11 percent were of a chemical nature and 34 percent were organisational in origin. The remaining errors, 1.3 percent of all tests performed, were transcription, chart reading and calculation mistakes.

In order to determine our error rate, a study was undertaken in our routine section, excluding special test areas such as toxicology, steroids and protein analysis, intended to detect transcription, chart reading and calculation errors.

**Method**

After test results were recorded on the patient result forms by the staff, the forms were checked by the author against the worksheets to detect transcription errors. Also machine printouts were checked against the worksheets. All calculations not performed by a machine were re-calculated and all Auto-Analyzer chart peaks were re-read. Any error detected was recorded on a long sheet as: (a) type of err or, (b) nature of error, (c) date and time of occurrence, (d) work area concerned. The staff were not made aware of this study but any error detected was immediately brought to their attention.

| *Type of Error* | *Number* | * *of Total Tests* |
|---|---|---|
| Transcription | 61 | 0.25 |
| Calculation | 30 | 0.125 |
| Chart Reading | 26 | 0.105 |
| All Errors | 117 | 0.48 |

**Results**

After a three-week period during which, 24,275 patient test results were produced, the following errors were detected:

Some of the typical errors detected were:

1. Transcribing fasting glucose results as random, and vice versa.
2. Leaving out decimal points on results.
3. Transcribing results on wrong patient's report form.
4. Forgetting to dilute high concentrations of serum constituents.
5. Making arithmetic errors with electronic calculators.
6. Miscalculating peak heights.

**Discussion**

Using our department's 95 percent confidence limits for each test, 54 percent of the errors detected were statistically significant; however, a statistically significant error does not necessarily imply clinical significance. The clinical significance of our errors was not followed up as the significance would have to be considered in conjunction with the clinical setting in which the erronerous results occurred; that is, would the erroneous result alter treatment, lengthen the hospital stay or lead to further testing? It should be recognised however, that any error made could be serious and is not acceptable.

As our reporting system is mainly a manual operation, our division would obviously benefit from computerisation which would reduce manual clerical work and the error rate. Because manual operations are prone to errors we must be alert and vigilant. Staff must be notified if an

error has occurred, how and why it has occurred, what consequence the erroneous result could have and how it might be prevented from recurring. Furthermore, instructions in test procedures and laboratory calculations have to be written down and taught in a logical, simple and precise manner. Finally, the division should be organised so that all errors, chemical and non-chemical, are liable to be detected since all errors are potentially serious.

**Acknowledgments**

The author wishes to thank Dr H. Ford and Mr W. Eden for their help and support during this study and Mrs M. Pyett for her clerical assistance.

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# Estimation of HDL-Cholesterol In Serum

**R. M. Millard**

**Hamilton Medical Laboratory, Hamilton**

*Received for Publication, August 1978*

**Summary**

A method for the estimation of HDL-cholesterol by precipitation with phosphotungstate-magnesium chloride and an enzymatic analysis is described. The usefulness of HDL-cholesterol estimations as a possible screen for coronary heart disease is briefly discussed.

**Introduction**

An association between elevated total serum cholesterol levels and coronary heart disease (CHD) has long been recognised. This relationship is complex and multifactorial[8], and presents limitations in clinical usefulness. The observation that high levels of High Density Lipoprotein (HDL) are associated with low levels of CHD was known in 1951. However, very little interest was exhibited until 1975 when a review of epidemiological data was made[7] and it was proposed that low levels of plasma HDL would accelerate atherosclerosis and thus CHD. Experimental work appeared to support this hypothesis. Following this review the Framingham[2] and Tromsø[6] studies confirmed that HDL is an independent, inversely-related factor of CHD. There is a low incidence and prevalence of CHD amongst people with high levels of HDL, extending over various race, sex and age groups.

The High Density Lipoprotein class may be estimated in a number of ways such as by ultracentrifugation, electrophoresis and precipitation techniques. Ultracentrifugation techniques, traditionally the reference method, are time-consuming and costly (in terms of technical and equipment expense), so the various precipitation techniques were investigated. The method finally selected involved precipitation of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) fractions using a phosphotungstate-magnesium chloride mixture. The remaining HDL fraction may be estimated by measuring its cholesterol content using any of the cholesterol methodologies available. Because of the limitations imposed by the direct Liebermann-Burchard methodology available on the Technicon SMA 12/60 analyser, an alternative method for cholesterol analysis was desirable.

**Materials**

Stock sodium phosphotungstate solution was prepared by dissolving 10 g dodeca-tungstophosphoric acid (BDH-AnalaR) in 100 ml distilled water, adding 40 ml of 1.0 mol/l NaOH (BDH-AnalaR) and making up to a total volume of 250 ml with distilled water. Stock magnesium chloride solution was prepared by dissolving 101.66 g $MgCl_2 .6H_2O$ (BDH-AnalaR) in distilled water to a total volume of 250 ml.

Cholesterol analysis was performed on a Union Carbide Centrifichem series 300 Centrifugal analyser using the Hoechst-Calbiochem cholesterol SVR enzymatic kit.

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**Method**

a) *Precipitation*

A modified method of Burstein and Samaille (1960)[1] was used. Immediately before use, working precipitant was prepared by mixing 5.0 ml stock sodium phosphotungstate solution with 1.25 ml stock magnesium chloride solution. Precipitant (75 $\mu$l) was added to 0.5 ml of serum in a polystyrene tube and the mixture vortexed immediately for a minimum of 7 seconds. The tubes were stood in an ice-water mixture for 20 minutes then centrifuged at room temperature for 30 minutes at 1,500 g. The supernatant was decanted into an Auto-analyser cup.

b) *Cholesterol Analysis*

Cholesterol enzymatic reagent was reconstituted as instructed in the kit insert. The Centrifichem pipettor was set to sample 10 $\mu$l of supernatant, followed by a diluent wash of 40 $\mu$l and 250 $\mu$l of reagent. The terminal reaction was carried out at a temperature of 30°C in the centrifugal analyser and the optical density measured at 500 nm after 16 minutes. Values were multiplied by 1.15 to correct for the addition of precipitant. Assayed Ortho-Normal sera was used as the control.

**Results**

The enzymatic cholesterol method was linear over the range 0.1-10.0 mmol/l which amply covers the HDL-cholesterol values expected in routine screening. No significant interference by triglycerides up to a concentration of 8.0 mmol/l was noted. A standard deviation of 0.04 mmol/l at a cholesterol value of 1.50 mmol/l was obtained. Between-batch optimum coefficient of variation was 1.8 percent at 1.50 mmol/l.

**Discussion**

The low values (relative to total serum cholesterol) of serum HDL-cholesterol normally encountered meant that the direct Liebermann-Burchard methodology routinely available on our SMA 12/60 (with its predilution manifold) posed difficulties as these values are in the non-linear portion of the range[10]. In addition, selecting the appropriate calibration values for the control panel posed further problems. The alternative of developing an enzymatic method for the Union Carbide Centrifichem was attractive. Once the enzymatic method had been decided upon, the selection of an appropriate precipitation method became important. One study[5], comparing the separation of HDL by ultracentrifugation, heparin-$MnCl_2$ precipitation and phosphotungstate-$MgCl_2$ precipitation, reported no significant differences between the three methods, but noted that heparin preparations were not equally efficient in precipitation, and that hyperlipemic sera caused problems of incomplete precipitation using heparin-$MnCl_2$. In addition, manganous ions give falsely low cholesterol values using methodologies involving enzymatic reagents[4]. Inhibition of the Calbiochem enzymatic cholesterol reagent by manganous ions in the concentration used for precipitation was observed (unpublished data). Therefore, the phosphotungstate-$MgCl_2$ precipitation method was chosen as the most suitable for routine use.

**Conclusion**

The inverse relationship between serum HDL levels and the incidence of CHD opens the possibility that HDL can be used as a risk assessment for CHD[2,3,6]. One such survey[9] has already been performed in New Zealand confirming the trends reported by overseas studies. The method reported here presents a simple and rapid means for routine estimation of HDL-cholesterol.

**Acknowledgments**

The author gratefully acknowledges Hamilton Medical Laboratory and the Partners for the facilities provided.

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distribution of serotypes in various clinical specimens is shown in Table III. Types O8 and O14 were the most common accounting for 38 percent and 30 percent of strains respectively.

The *S. marcescens* strains comprised a wide variety of O and bacteriocine types, the majority of which showed more or less random distribution. Using three criteria—O type, bacteriocine type and antibiotic resistance pattern—two episodes of cross-infection were detected. These involved four and two patients respectively in two different hospitals.

Table III.

—Distribution of *Serratia marcescens* O serotypes

| | Number of strains isolated from: | | | | |
|---|---|---|---|---|---|
| Serotype | Urine | Wound Swab | Sputum | Others | Total |
| O3 | | | | 1 | 1 |
| O4 | | | 1 | 1 | 2 |
| O5 | | 1 | | 2 | 3 |
| O6 | 3 | 1 | 1 | 1 | 6 |
| O8 | 19 | 3 | | 1 | 23 |
| O12 | | | 2 | | 2 |
| O14 | 7 | 4 | 4 | 3 | 18 |

## Discussion

*Serratia* accounted for a small but significant proportion of *Enterobacteriaceae*-like strains isolated from clinical specimens received by the diagnostic laboratory at St Bartholomew's Hospital. Our *Serratia* isolation rate was low in comparison with hospitals in the U.S.A. but was approximately twice that reported by Black *et al.* (1971)[2], who conducted a similar survey in Scotland and considerably greater than that reported by Hedges *et al.* (1975)[6], who recorded only one or two *Serratia* isolations per annum in a laboratory handling about 60,000 clinical specimens per year. The generally accepted view, that *Serratia* is a rare, nosocomial pathogen in Britain was certainly not confirmed by the present study. There may have been a recent increase in the incidence of *Serratia* in British hospitals although the possibility that this organism has been present in the hospital community for some time, but has previously gone unrecognised, cannot be excluded. The majority of nosocomial strains are non-pigmented and have no obvious morphological distinguishing features. Thus, the limited number of identification tests employed in most hospital laboratories may fail to separate members of the *Klebsiella-Serratia-Enterobacter* group. A 2-stage screening procedure for the identification of *Serratia* spp. proved to be both useful and economical in the present study.

In agreement with American authors (Wilfert *et al.*, 1968[13], Schaeffler *et al.*, 1971) our strains exhibited a high degree of antibiotic resistance and all were multiply resistant. No gentamicin or tobramycin resistant strains were isolated, although the incidence of aminoglycoside resistant *S. marcescens* has reached frightening proportions in some American hospitals. Meyer *et al.* (1976)[10] reported resistance to gentamicin, tobramycin and sisomicin in 50 percent of *Serratia* strains isolated over a one-year period in a Los Angeles hospital.

The epidemiology of nosocomial *Serratia* spp. has not been fully elucidated and, in the present study, no attempt was made to locate their source. However, the wide variety of sero-bacteriocine types indicated that the majority of infections were sporadic and cross-infection was not a major mode of spread. Previous investigations have shown rectal and pharyngeal carriage to be of little importance but that outbreaks of *Serratia* infections are often associated with contaminated irrigating fluids, breathing machines, catheters and suction tubing (McCormack *et al.*, 1966[8]; Wilkowske *et al.*, 1970[14]; Whitby *et al.* 1972[12]).

## Conclusion

There is no doubt that *Serratia* is capable of causing nosocomial infections of a severe nature and in addition it is usually highly resistant to antibiotics. *S. marcescens* has become almost as infamous as *Pseudomonas aeruginosa* in American hospitals and this is sufficient cause for concern about the future of hospital acquired infections in other countries.

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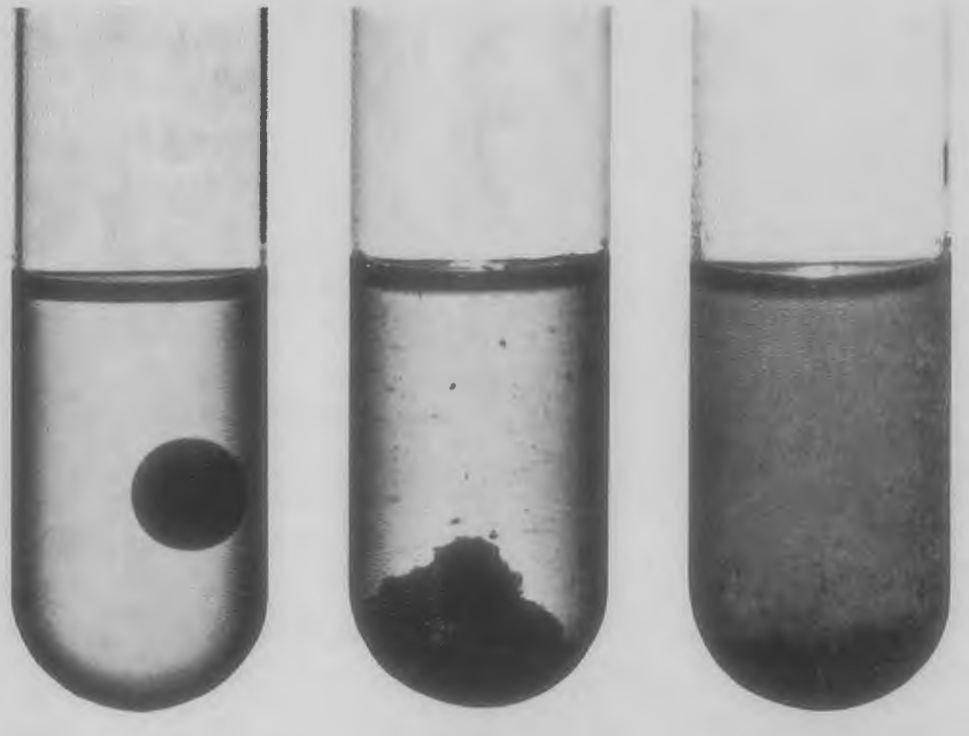

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# Chemotherapy of Primary Amoebic Meningo-Encephalitis (PAM)

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*A Paper read to the NZIMLT Conference Nelson, 1978*

**Summary**

The treatment of the rapidly fatal disease Primary Amoebic Meningo-encephalitis (PAM) has to date been successful in only one case. Further screening of chemotherapeutic agents is necessary and it appears that the best regimen for the treatment of infection due to both *Naegleria* and *Acanthamoeba* would be the use of synergistic combinations of drugs.

**Introduction**

The ability of free-living amoebae to cause disease in man was first recognised by Fowler and Carter in 1965.[6] In man and animals they have been responsible for a multitude of diseases, ranging from chronic illnesses such as respiratory infections and blindness to the very acute disease of Primary Amoebic Meningo-encephalitis (PAM). The amoebae belong to the genera *Naegleria* and *Acanthamoeba*.

Although a variety of drugs has been used to combat infections caused by either *Naegleria* or *Acanthamoeba*, treatment has been unsuccessful in all but one case actually verified by isolation. Anderson and Jamieson (1972)[1] reported that intensive treatment with the fungicide amphotericin B resulted in the survival of a patient infected with *N. fowleri*. However, amphotericin B is nephrotoxic in 80 percent of patients (Goodman and Gilman, 1970).[7]

Amphotericin B is primarily an antifungal agent. Two other antifungal agents, 5-fluorocytosine and miconazole have been shown to have *in vitro* activity against the amoebae (Stevens and O'Dell, 1974[13]; Casemore, 1970[2]; Nagington and Richards, 1976[10]).

Since amphotericin B has been shown to potentiate the action of antibiotics (Kwan *et al.*, 1972[9]) the possibility of synergism between amphotericin B and either 5-fluorocytosine, rifampicin or tetracycline was investigated.

Polymyxin B sulphate, like amphotericin B, is a membrane active compound, which has been shown to have *in vitro* activity against *Acanthamoeba* spp. (Nagington and Richards, 1976[10]; Casemore, 1970[2]; Duma and Finley, 1976[5]). However the concentrations required are prohibitive for systemic use. The possibility of combining polymyxin B with either 5-fluorocytosine or tetracycline was investigated.

**Materials and Methods**

Amoebae:

*Naegleria gruberi* (PL200f)—non-pathogen
*Naegleria fowleri* (MsT)—pathogen
*Acanthamoeba castellani* (1501)—non-pathogen
*Acanthamoeba culbertsoni* (A-1)—pathogen.

*Naegleria* spp. were maintained in the semi-defined medium CGHV (Cursons *et al.*, 1978[4]) and *Acanthamoeba* spp. in CGHVS. The pathogenic strains were incubated at 37°C and the non-pathogenic strains at 30°C on a rotary gyroshaker (150 rpm).

Solutions of the test compounds were freshly prepared according to the manufacturer's instructions and control tests were made of all diluents.

**Table 1.—*In vitro* susceptibility of *Naegleria* spp. to six chemotherapeutic agents.**

| | Range of concentrations | *N. gruberi* (PL200f) non-pathogen | | *N. fowleri* (MsT) pathogen | |
|---|---|---|---|---|---|
| | tested | MIC | MAC | MIC | MAC |
| Amphotericin B | 0.108-1.08 | 0.215 | 0.54 | 0.215 | 0.54 |
| 5-fluorocytosine | 100-1000 | >1000 | — | >1000 | — |
| Rifampicin | 10-500 | 500 | — | 500 | — |
| Tetracycline | 10-400 | 400 | — | 400 | — |
| Polymyxin B sulphate | 6.35-63.5 | >63.5 | — | >63.5 | — |
| Miconazole nitrate | 10-100 | 50 | 100 | 10 | 50 |

**Table II.—*In vitro* susceptibility of *Acanthamoeba* spp to six chemotherapeutic agents.**

| | Range of concentrations | *A. castellani* (1501) non-pathogen | | *A. culbertsoni* (A-1) pathogen | |
|---|---|---|---|---|---|
| | tested | MIC | MAC, | MIC | MAC |
| Amphotericin B | 0.54-5.4 | >5.4 | — | >5.4 | — |
| 5-fluorocytosine | 10-250 | 10 | 250 | 10 | >250 |
| Rifampicin | 10-500 | 500 | — | 500 | — |
| Tetracycline | 10-400 | 400 | — | 400 | — |
| Polymyxin B sulphate | 6.35-63.5 | >63.5 | — | >63.5 | — |
| Miconazole nitrate | 10-100 | >100 | — | >100 | — |

Concentrations in $\mu g.cm^{-1}$.

Minimum inhibitory concentration (MIC)—Lowest concentration of test compound which produced obvious reduction in the number of trophozoites over 96 h. Minimum amoebicidal concentration (MAC)—Lowest concentration after exposure to which no living trophozoites could be found after 96 h.

Tests were carried out in small screw-cap bijous in a total volume of 2.0 $cm^3$. The inoculum used was adjusted to give a final concentration of 2-3 x $10^5$ amoebae $cm^{-3}$. Dilutions of the test compounds were made so 0.5 $cm^3$ gave the required final concentration the ranges of which are given in Tables I and II. Samples were withdrawn at 24, 48, 72 and 96h and the surviving amoebae were counted on a modified Fuchs-Rosenthal bright-line haemocytometer. Viability tests were carried out by plating on amoeba saline (AS) agar (Page, 1976[11]) spread with a lawn of *Enterobacter cloacae*. Synergy experiments were carried out using a checkerboard titration strategy.

**Results and Discussion**

The results shown in Tables I and II demonstrating the *in vitro* effectiveness of amphotericin B against the amoebae, are in agreement with most published reports, with *Naegleria* spp. being susceptible at levels attainable in the body. *Acanthamoeba* spp. are susceptible only at prohibitive levels. Casemore (1970)[2] and Cerva (1972)[3] found that *Acanthamoeba* were inhibited at 100-125 $\mu gcm^{-3}$ amphotericin B.

Duma and Finley (1976)[5] found that the imidazole derivatives clotrimazole and miconazole were also effective against *Naegleria* but their effect was not as rapid as that of amphotericin B. Although *in vitro* tests of clotrimazole looked promising Jamieson (1975)[8] showed that mice inoculated with *N. fowleri* were not protected from infection by doses of 100 mg clotrimazole/kg/day given for five days after inoculation. The mice had serum levels far in excess of minimum amoebicidal concentrations within 32h of inoculation.

No *in vivo* tests have been carried out with miconazole but its greater penetrability of the blood/brain barrier would make such studies worthwhile.

Table II shows that the non-pathogenic *Acanthamoeba* sp. is more susceptible to 5-fluorocytosine (5-FC) than the pathogenic species

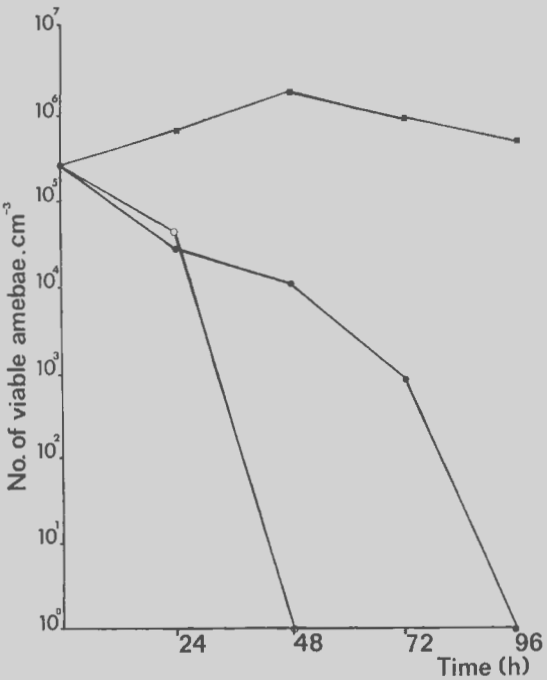


Figure 1.—Synergistic activity of Amphotericin B and Rifampicin against *N. fowleri* (MsT). Symbols: ●, 0.54 μg.cm⁻³ Amphotericin B; ■, 100 μg.cm⁻³ Rifampicin; ○, combined effect of 0.54 μg.cm⁻³ Amphotericin B + 100 μg.cm⁻³ Rifampicin.

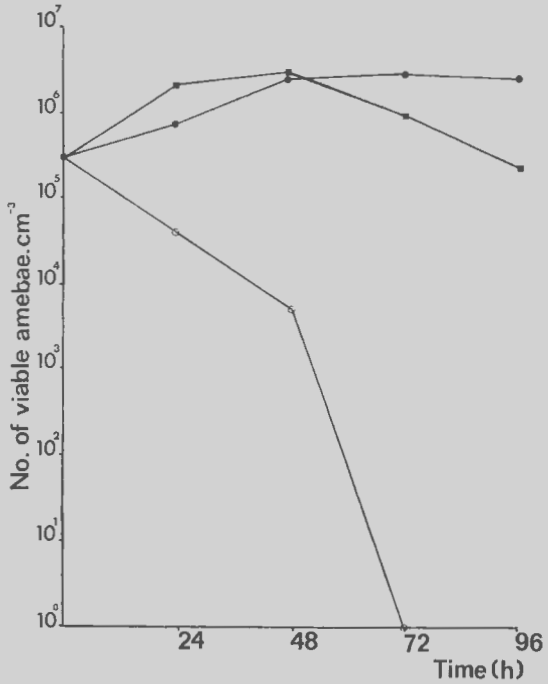


Figure 2.—Synergistic activity of Amphotericin B and Tetracycline against *N. fowleri* (MsT). Symbols: ●, 0.108 μg.cm⁻³ Amphotericin B; ■, 100 μg.cm⁻³ Tetracycline; ○, combined effect of 0.108 μg.cm⁻³ Amphotericin B + 100 μg.cm⁻³ Tetracycline.

*A. culbertsoni* (A-1). Similar results were found by Stevens and O'Dell (1974)[13]. In mouse protection studies they found that 5-FC promoted survival when treatment was initiated at the onset of amoebic infection but the failure of the drug to protect animals when treatment was begun 24h post-infection indicates that it may be of limited usefulness in human *Acanthamoeba* infections.

Rifampicin and tetracycline were reported by Thong *et al.* (1977)[14] to significantly inhibit growth of *N. fowleri*. In an effort to reduce the concentration of amphotericin B required to sterilize the media it was used in conjunction with either rifampicin or tetracycline. The results obtained are shown in Figures 1 and 2. The combination of 100 μg.cm⁻³ rifampicin with 0.54 μg.cm⁻³ amphotericin B halved the time required to completely sterilize the media.

Figure 2 shows the considerable synergistic activity of amphotericin B and tetracycline. For amphotericin B to be effective alone a concentration of 0.54 μg.cm⁻³ is required and tetracycline has inhibitory activity at 400 μg.cm⁻³. When used together the concentrations needed are only 0.108 μg.cm⁻³ and 100 μg.cm⁻³ respectively.

Thong *et al.* (1978)[15] have since shown that combined therapy of amphotericin B and tetracycline increased the survival of mice when treatment was begun 24 h after inoculation with pathogenic *Naegleria*.

The lack of susceptibility of *Acanthamoeba* spp. to all chemotherapeutic agents tested is very disturbing. But the use of a combination of drugs may again prove the best approach.

Figure 3, which shows the results obtained by using the membrane-active agent Polymyxin B sulphate in combination with 5-fluorocytosine demonstrates the considerable increase in effectiveness with a synergistic mixture is used. Polymyxin B sulphate has inhibitory activity at levels >63.5 μg.cm⁻³ when used alone. The MAC at 96 h for 5-FC is >250 μg.cm⁻³. When used together complete sterility of the medium is achieved with 63.5 μg.cm⁻³ Polymyxin B sulphate + 50 μg.cm⁻³ 5-FC.

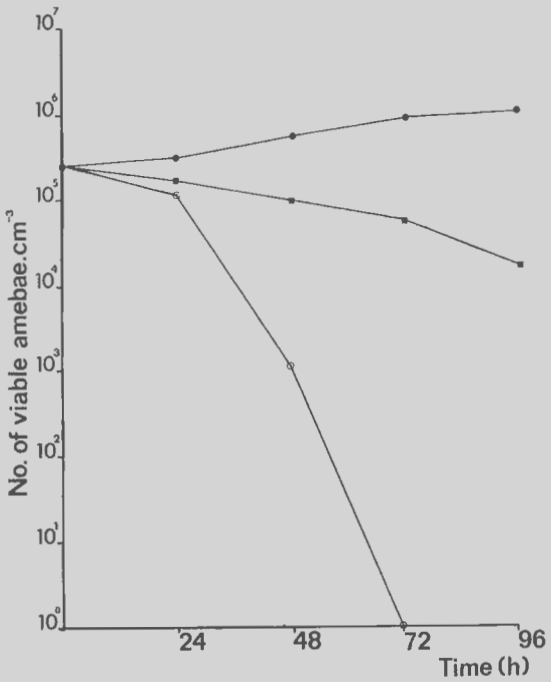


Figure 3.—Synergistic activity of Polymyxin B sulphate and 5-Fluorocytosine against *A. culbertsoni* (A-1). Symbols: ●, 63.5 μg.cm⁻³ Polymyxin B sulphate; ■, 50 μg.cm⁻³ 5-Fluorocytosine; ○, combined effect of 63.6 μg.cm⁻³ Polymyxin B sulphate + 50 μg.cm⁻³ 5-Fluorocytosine.

**Conclusions**

From the results of studies to date it would appear that the best approach to the treatment of infections due to both *Naegleria* and *Acanthamoeba* spp. would be the use of synergistic combinations of chemotherapeutic agents. For each species the use of a different membrane-active compound appears to permit entry of an otherwise ineffective agent.

**Acknowledgments**

We would like to acknowledge Roche Products, Ethnor Pty Ltd., the Medical Research Council and the N.Z. Health Department for financial support of this work.

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# Book Reviews

**MCO Tutor for Students of Clinical Chemistry.** Derek Knowles, FIMLS, BA. Published by William Heinemann Medical Books Ltd, London, 1978. 159 pages, £3.75 (U.K.).

This little book was specifically prepared for the IMLS Fellowship examination in clinical chemistry by a practising technologist. Multiple choice questions are apparently used in this examination and the intention is to assist in answering such questions. They do simplify marking and avoid subjectivity. They are not greatly favoured here, and indeed could only constitute part of an examination as the essay type of question is required to evaluate the candidate's capacity for coherent thought and logical expression.

The book is arranged under 15 chapter headings and these include inborn errors, vitamins/trace metals, haemoglobin/porphyrin, toxicology and instrumentation.

The nature of the questions is basic rather than exotic. There is no attempt to probe into recent advances in the subject. The first page for example relates to choosing a pH given the hydroxide ion concentration, relative concentrations of acetate and acetic for a given pH, the definition of the Henderson-Hasselbach equation and consistent data for normal arterial blood. It is however reasonably up to date; EMIT gets a mention in the toxicology section.

I think an NZCS (medical) student could be expected to answer the majority of the questions.

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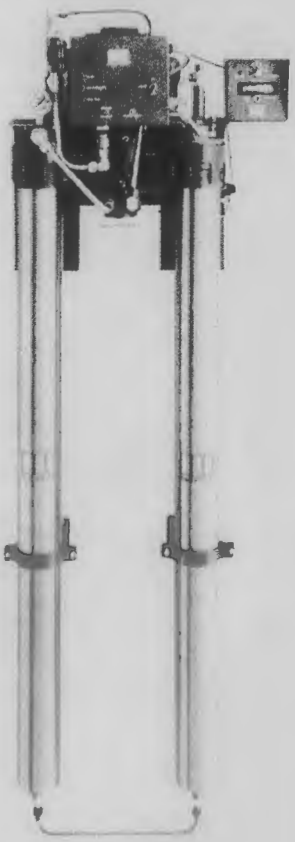

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I believe it is axiomatic that a book of this nature is no substitute for a textbook, nor I hasten to add is it intended to be and apart from gaining familiarity with the presentation of MCQ it can be used as an adjunct to textbook learning by providing a means of assessment of the topics covered. The need to select specific meanings certainly sharpens perceptions. While the questions are of necessity arbitrary this is equally true of examination questions. R.D.A.

**Biochemistry in Schematic Perspective.** J. Musil, O. Novakova and K. Kunz, 1977. First English Edition. Avicenum, Czechoslovak Medical Press, Prague. 182 pages. Illustrated.

This edition was produced to commemorate the 25th anniversary of Calbiochem. A limited number are available for sale at $15.00 from Calbiochem-Behring.

This is essentially a basic text in biochemistry. Practically every page contains a schematic illustration or table. Use is made of a two colour system both for the illustrations and for headings in the text, with pleasing effect.

The implication in the foreword is that the book has been available in Czechoslovakia and has now been translated. This has been done in a very competent fashion and there are few infelicities of style or grammar.

The chapter headings are: proteins, enzymes, amino acids, glycolysis, lipids and steroids, the citric acid cycle, porphyrins, the cell, cell membranes and transport, mitochondria respiration and phosphorylation, the nucleus genetic information and transmission, ribosomes polysomes and protein synthesis, principles of metabolic control.

This compact illustrated treatise presents the biochemical pathways in an illuminating and arresting fashion and could be a worthy acquisition for those concerned with learning and teaching biochemistry. R.D.A.

**Environmental Health Criteria 6: Principles and Methods for Evaluating the Toxicity of Chemicals. Part 1.** Published under the joint sponsorship of the United Nations Environment Programme and the World Health Organization, Geneva, World Health Organization, 1978 (ISBN 92 4 154066 4). 272 pages. Price: Sw. fr. 28.-, $US15.40. French edition in preparation obtained from the Government Bookshops.

The sixth publication in the series on Environmental Health Criteria is concerned with the principles of toxicity testing and the procedures involved. More than 50 distinguished experts from some 11 countries and the staff of a number of WHO Collaborating Centres co-operated with the World Health Organization in the preparation of this work.

The work has been divided into two separate publications. The first part now under review contains the broad principles and more general aspects of toxicity testing; the second part (in preparation) will systematically cover some more specialized procedures for safety evaluation and will consider factors that may modify the outcome of toxicity testing and evaluation. Individual chapters in the first part deal with general aspects of toxicity evaluation, including definitions, dose-effect and dose-response relationships, interpretation of laboratory data, ethical considerations, and establishment of environmental health standards; factors influencing the design of toxicity studies; the principles and design of tests for acute, subacute and chronic toxicity; chemobiokinetics and metabolism; morphological studies; inhalation exposure; and carcinogenicity and mutagenicity tests and their significance.

Each chapter is followed by an extensive list of references, amounting in total to about 800 citations.

The general approach has been to present the underlying scientific principles, to evaluate the utility, strengths and weaknesses of various methods and procedures, in order to help the reader to select the most suitable technique for a specific purpose, though not prescribing standard tests.

The work, compiled in close collaboration with the International Agency for Research on Cancer, is published under the joint sponsorship of the United Nations Environment Programme and WHO.

The publication will be of interest to toxicologists, oncologists, pathologists, occupational and public health workers, departments of the environment and of health protection, and national regulatory agencies.

WHO CIRCULAR

**Hospital Hygiene.** 2nd Edition, 1978. Isobel M. Maurer, B.Sc., Dip.Ed. (London.) Published by Edward Arnold Ltd, London. 138 pages, illustrated. Obtained from the Publishers, 41 MMaddocx St, London W.I. Price £3.95 (U.K.).

The introductory chapters of this book deal with hospital infection, its cause and control and a summary of sterilisation methods including heat, gas and radiation.

However, the emphasis is on disinfection, and methods of pasteurisation, pasteurising equipment, laundering, dishwashing and general cleaning of the hospital environment are described in greater detail.

Three chapters are devoted to chemical disinfectants. The advantages and disadvantages of various groups are outlined the factors affecting their behaviour, and methods of testing disinfectants are discussed, namely the Chick-Martin and the more recent Kelsey-Sykes tests. Particularly relevant for the hospital laboratory worker is the chapter concerned with the use of and checking in-use chemical disinfectants. If more information is required on a particular aspect, a comprehensive list of references is included at the end of each chapter.

While the principles of hygiene are the same the world over this second edition is of added value to readers in Australia, New Zealand and South Africa as it includes local standards for sterilisers and local brands of disinfectants in these countries.

I find that this is a worthwhile and also readable book made interesting by the addition of many photographs and diagrams. It is also not devoid of humour. In one cartoon, a man from the complaints department answers "Good heavens madam, of course our product was laboratory tested . . . it failed miserably but it was definitely tested!"

In summary this book would be a worthwhile addition to most hospital department bookshelves.

M.J.

**Textbook of Immunology. An Introduction to Immunochemistry and Immunobiology.** James T. Barrett, 3rd Ed. 1978. Published by C. V. Moseby Co. St Louis and obtained from N. M. Peryer, C.P.O. Box 833, Christchurch. 505 pages, 242 illustrations, paperbacked. Price $NZ20.95.

It appears from the preface, that although this book resulted from a specific course in immunology, it was written as an introduction to cover a wide range of disciplines and does make certain demands and assumptions of prior knowledge on the part of the reader.

It is a large book divided into three sections. 1. Foundation of Immunology. 2. Serological Reactions. 3. *In vivo* Aspects of Immunology.

The first section starts with the inevitable historical introduction ornamented by portraits of the hirsute pioneers and goes over all the usual introductory material of immunocytology, antigens, immunoglobulins and biological aspects of the immune response. As far as possible the reactions are described on a structural or macromolecular basis. For example the aminoacid sequence of common antigenic determinant sites related to hetero-immunisation are detailed. The aminoacid sequence or molecular structure of many substances are described including the "J" or joining chain which now appears to have the function of initiating polymerisation of secretory IgA and IgM. There is a sound treatment of the hapten modification of antigens the production of specific antibodies and their use in quantitation of biological compounds.

The second section covers serological reactions and the chapters deal with antigen antibody reactions, precipitation, agglutination and haemagglutination, and a very long chapter on complement. Techniques are described in principle rather than practical detail. Radioimmune assay, enzyme immunoassay, fluorescent and ferritin tagged antibodies are mentioned. The description of the early fractionation studies of complement were particularly illuminating describing the four fractions originally separated. At least 19 are now recognised to participate in this highly complex system.

The third section considers phagocytosis, immunity, allergy and hypersensitivity, IgG and T cell dependent allergies, transplantation immunity, tumour immunology and autoimmune disease.

A great deal of information is given about the cellular aspect of immunity. A group of diseases specifically affecting phagocytes including immunodeficiencies are described. In the chapter on

T cell dependent allergy the lymphokines and other extracellular factors are described at length.

Some of the chapters conclude with an imaginary case history illustrating a relevant point, sometimes contrived to the point of risibility!

There are three appendices. 1. A review of macromolecular chemistry. Scarcely adequate to cover the knowledge required to follow this text. 2. Readings in immunology. These are quite interesting. They consist of seminal discoveries in immunology provided in the form of the original articles. 3. There is a glossary.

In conclusion, I found this a weighty book both in size and treatment of the subject. If it says little new. It leaves few stones unturned. It is not dear for a book of this size. R.D. Allan.

**A Clinical Companion to Biochemical Studies.** Victor Schwarz. Published by W. H. Freeman and Co., California. Obtained in N.Z. from N. M. Peryer Ltd, Christchurch. 114 pages. $NZ9.40.

Any book which might promote enthusiasm for biochemistry in the pre-clinical medical student demands close attention and tentative support. Dr Schwarz has good intentions and deserves praise for his attempt to perform that near miracle; unhappily, this volume is unlikely to live up to the hopes of its author.

The text is arranged into 22 case histories, each featuring a clinical presentation, a few salient physical findings and the results of a limited number of laboratory investigations. The cases have been "synthesized" from a mixture of sources and many have been used in student teaching at the University of Manchester. The author has been unable to resist the temptation to use many "inborn errors" as example of biochemical diseases. Whilst these (in their classical presentation) show with great clarity the possible multiple consequences of a single faulty enzyme, the overall effect is to suggest to the reader that these rare disorders are relatively common in everyday clinical practice. This, plus the presentation of detailed "biochemistry" and the scant regard given to "chemical pathology" severely limit the usefulness of the book. It may be a companion, but it is certainly not an adequate guide to the clinical use of biochemistry in diagnosis and assessment. To be fair to the author, he states that he did not attempt to present disorders because of their importance or frequency. Nonetheless, the overall emphasis on the rare and exotic, and the omission of the commonplace, plus the rather too frequent errors in "chemical pathology" would probably make this book unsuitable for medical students.

The book is well presented, in an attractive paper back style, with several photographs and numerous diagrams of biochemical pathways. A minor annoyance is the lack of information in the legends to the colour-plates.

In summary, this book is of considerable interest as a bold attempt to revitalise the classical biochemistry studied in medical schools—but an attempt that is largely unsuccessful.

C. Lovell Smith.

# Abstracts

**Contributors: E. R. Crutch, Shirley Gainsford, Ainslie Langford, N. Langford and L. M. Milligan**

## Clinical Biochemistry

**Comparison of Alkaline Phosphatase Isoenzymes Determined by an Inhibition Method and by Electrophoresis.** Duukka, R. (1978), *Clin. Chim. Acta.* **85,** 111.

An inhibition procedure suitable for routine determination of alkaline phosphatase isoenzymes has been adapted for use with a fast kinetic analyser, System Olli 3000. This method is compared with electrophoretic separation of alkaline phosphatase isoenzymes. The results obtained indicate that AP-urea/AP ratio may be used to differentiate between patients with bone and liver disease. It is also possible to estimate relative bone

and liver isoenzyme activities from this ratio using two equations. —A.L.

**Regan Variant Alkaline Phosphatase in Gastrointestinal Carcinoma.** Crofton, D. and Smith, A. F. (1978). *Clin Chim. Acta.* **86,** 81.

A fast moving alkaline phosphatase band or polyacrylamide gel electrophoresis has been found in six patients with carcinoma of the liver and gastrointestinal tract. This Regan variant has been reported in hepatocellular carcinoma and is now reported in this paper to appear in sera of patients who have neoplasms in a variety of primary sites in the gastrointestinal tract. —A.L.

**Comparison of the EMIT Test with High-Performance Liquid Chromatography for the Determination of Phenobarbital in Serum.** Dellamonica, C., Bory, C., Battassat, P. and Lahet, C. (1978), *Clin. Chem. Acta.* **86,** 1.

The new enzyme immunoassay technique (EMIT) is compared with high-performance liquid chromatography used for determining phenobarbital in serum. The reliability characteristics are comparable. The enzyme immunoassay technique is less specific but more practicable. —A.L.

**Acute Pancreatitis, Hyperlipemia and Normal Amylase.** (Washington University Case Conference). Edited by: J. H. Ladenson and J. M. McDonald. Presenter: J. M. McDonald. Discussants: D. E. Bruns, J. H. Ladenson and J. C. Mauck (1978), *Clin. Chem.* **24,** 815.

This case discusses the biochemical findings in acute pancreatitis and the laboratory role in diagnosis and management of such patients. It illustrates a major problem in the use of amylase determination in patients with acute pancreatitis: normal serum amylase activity in the presence of hyperlipemia. —A.L.

**Evaluation of Fecatest, a New Guaiae Test for Occult Blood in Faeces.** Adlercreutz, H., Liewendahl, K. and Virkola, P. (1978), *Clin. Chim.* **24,** 756.

This test for occult blood in faeces is based on guaiae tests, and has been evaluated using specimens from hospitalized patients. All steps of test are performed in a plastic case which reduces risk of contamination of laboratory personnel. Results showed Fecatest to be less sensitive than benzidine test and the Hematest but more sensitive than the Hemoccult and guaiae extraction tests. —N.L.

**New Ultraviolet (340nm) Method for Assay of Uric Acid in Serum or Plasma.** Trivedi, R., Rebar, L., Desai K., and Strong, L. (1978). *Clin. Chem.* **24,** 562.

This enzymatic method for assay of uric acid at 340nm elimiates several disadvantages of the colorimetric and enzymatic methods now in use. Uric acid is oxidised to allantoin and hydrogen peroxide. The peroxide is reacted with ethanol in the presence of catalase to form acetaldehyde and water. Acetaldehyde is reduced by NADH in the presence of alcohol dehydrogenase to ethanol and the decrease in absorbance at 340nm caused by oxidation of NADH is directly proportional to the concentration of uric acid. N.L.

**Enzymatic Determination of Blood Ethanol, with Amperometric Measurement of Rate of Oxygen Depletion.** Cheng, F. S., and Christian, G. D. (1978), *Clin. Chem.* **24,** 621.

A method using the Beckman Glucose Analyser for measurement of blood alcohol is described. NADH is produced from a reaction between NAD and Alcohol in the presence of alcohol dehydrogenase. The NADH produced is oxidized by horseradish peroxidase. The rate of oxygen depleted is measured by the oxygen sensing electrode. The results obtained were compared with those obtained on the same samples tested by gas chromatography and a U.V. enzyme kit method showing good correlation. —N.L.

**A Simple Radioimmunoassay for Plasma Cortisol.** Seth, J. and Brown, L. M. (1978), *Clin. Chim. Acta.* **86,** 109.

A simple RIA method for cortisol is described in which analyses can be completed in four hours. Extraction of serum or plasma samples is not required and solid coupled cortisol antibodies are used. The technique is claimed to be more simple to use than the fluorimetric, competitive protein binding, and other RIA methods now used. —N.L.

## Haematology

**Disseminated Intravascular Coagulation: A Review,** Hamilton, P.J., Stalker, A.L. and Douglas, A.S. (1978), *J. clin. Path.* **31,** 609.

This paper reviews DIC under the following headings: Nomenclature, Definition and Pathophysiology, Occurrence. Laboratory Studies and diagnosis, Histopathology and management of the patient. It is a succinct review in 10 pages and includes over 80 references. —E.R.C.

**A simpler and less Expensive Approach to Platelet Counting with Improved Precision using the Technicon Autocounter.** Laver, M.M., and Howel, M.F. (1978), *J. clin. Path.* **31,** 390.

The authors have designed a new and simple manifold to replace the complex manifold, which also decreases the sample aspiration by 73 percent and decreases the urea reagent used by 33 percent. They have also improved the peaks by introducing air and urea reagent at the top of the probe. Finally, they have eliminated the troublesome wash valve and at the same time decreased the amount of urea by a further 22 percent.

**Antithrombin 111. Theory and Clinical Applications.** Seegers, W.H. (1978), *Am. J. clin. Pathol,* **60,** 367.

This lecture discusses antithrombin 111, which is one of the main inhibitors in the blood coagulation mechanisms. —E.R.C.

**Cytochemical Applications in Haematology, with Particular Reference to Acute Leukaemias: A. Review.** Scott, C.S. (1978), *Medical Laboratory Sciences,* **35,** 111.

The role of cytochemistry in haematology is critically reviewed, with emphasis given to interpretation of cytochemical findings in normal and abnormal cell lines. —E.R.C.

**A Rapid Turbidimetric Determination of Fibrinogen Degradation Products (FDP).** Verbruggen, H.W. and Wessels, J.M.C. (1978), *Thrombo Haemostas. (Stuttg)* **39,** 12.

A rapid and simple turbidimetric determination of fibrinogen degradation products is described. This method is based on the increase of the turbidity due to the formation of precipitating antigen-antibody complexes after addition of rabbit antihuman fibrogen antiserum to human serum. The increase in turbidity correlates very well with results obtained with the haemagglutination inhibition technique and the reproducibility appears to be quite good. —E.R.C.

**A modified Sedimentation Method for Counting Platelets in Blood.** Archer, R.K., Allen, B.V. and Baldwin, C. (1978), *Br. J. Haemat.* **38,** 401.

A sedimentation method for counting human blood platelets is discussed which uses fluid of sp. gr. 1.057 at an osmolarity of 320 milliosmols. The fluid is a mixture of Lymphoprep and Isoton II. After centrifugation at or about 200 g platelets are counted on an electric particle counter.

—E.R.C.

**Ferritin: Structure, Biosynthesis and Role in Iron Metabolism.** Munro, H., and Linder, Maria C. (1978), *Physiological Reviews* **58,** 317.

This is a long (80-page) review of ferritin. The sections include structure of ferritin, assay, biosynthesis and degradation and role in iron metabolism. —E.R.C.

**Infectious Mononucleosis in the Older Patient.** Carter, J.W., Edson, R.S. and Kennedy, C.C. (1978), *Mayo Clin. Proc.* **53,** 146.

Six cases of infectious mononucleosis in patients more than 60 years old were reviewed. The geriatric patient with infectious mononucleosis often presents with nonspecific constitutional symptoms, without exudative pharyngitis. The disease may present as fever of obscure origin or mimic chronic lymphocytic leukaemia in this age group. Diagnosis is established through characteristic findings on haematologic and serologic studies. —E.R.C.

**Demonstration of the Fc Receptor of Blood Cells by Soluble Peroxidase-anti-Peroxidase (P.A.P.) complexes.** Huhn, D., Andrewa, P., Rodt, H., Thiel, E. and Eulitz, M. (1978), *Blut* **36,** 263.

The Fe Receptor of normal human leucocytes of Chronic Lymphatic Leukaemia cells and of Hematopoietic cell lines were demonstrated with soluble Peroxidase-anti-Peroxidase (P.A.P.) complexes. The biological significance of Fc receptors in different cells is still open to discussion.

—L.M.M.

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# Directions for Contributors

Original papers on topics related to medical laboratory science will be considered for publication. The original manuscript and one copy are required. Manuscripts should be typed on one side of the paper only, have 1" margins and be double spaced. Give the author's name with initials or one first name if desired. Indicate the address of the laboratory where the work was carried out. Only use capitals where grammatically indicated and not for headings. Underline only 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

Figures; cover graphs, photographs and drawings. The latter should be in black ink on stout paper. They should be about twice the size of the intended reproduction. Number consecutively with arabic numerals (1, 2, etc.,) and identify on the back. Legends are typed on a separate sheet.

Tables are typed separately and numbered with roman numerals (I, II etc.).

## 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 '1')
*Mass:* kg, g, mg, μg, ng, pg
*Mass concentrations:* mol/litre, mmol/litre, μmol/litre, nmol/litre.
*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 'specfic gravity')
*Clearance:* litre/s, ml/s

**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 words 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 should be listed alphabetically by author, at the end of the article and numbered in order. All author's names must be listed with initials; year of publication in brackets; journal title abbreviated accordingly to the World List of Scientific Periodicals (In general nouns have capitals, adjectives do not and conjunctions are omitted. Previous journals may be consulted). The title is underlined to indicate italics. This is followed by the volume number underlined with a wavy line to indicate bold type and finally the first page number.

Citations in the text are given the author's name using et al. if more than one, the year and the reference number as a superscript. Thus: Lowe *et. al.* (1978)[1]

## 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 at cost price, (minimum 50), from The General Printing Department, Otago Daily Times, Box 181 Dunedin. An official order is required and must be received prior to publication. Orders may be submitted when returning the proofs.

# *CONTENTS*

**Articles**