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T. H. Pullar Memorial Address 1980

The Journal

R. D. Allan
Journal Editor, Diagnostic Laboratories, Dunedin Hospital

The Inaugural Thomas Pullar address was delivered in Christchurch in 1967. Dr Pullar had died the previous year. Many members may wonder why the Institute wished to honour his memory in this fashion. Well I was about during his lifetime and although my acquaintance with him was slight, I remember the regard in which he was held by his colleagues in the laboratory. What emerged was simply a man secure in himself and kindly disposed towards us and our aspirations. This may not seem all that remarkable or unusual, but at that stage of our development which was characterised by a certain degree of patronage on the one side and some forelock tugging on the other, it was quite remarkable. Suffice to say that he helped us all he could, particularly in the field of education. I would certainly be prepared to include him in that select band featured in the November issue of the Journal in 1977 under the heading, 'Friends of the Institute'. As this would make a grand total of three and I would not wish to be thought ungracious if not downright churlish, I must hasten to add I could think of one or two more.

The original intent was to regard this address as a keynote address which to my mind implies a scientific content relating to a topic of current interest. The first Thomas Pullar address was just such an address. It was given by Dr F. H. Gunz who was the Haematologist at Christchurch at that time. It was a well illustrated and thorough review of cytogenetics, a subject which was quite new then. I really enjoyed it but it turned out to be virtually the first and the last paper of this nature to be given on this occasion. My first reaction to the invitation to give this address was to choose a scientific topic. There are plenty of them in the burgeoning field of immunology and in the arrival of the future in the form of microprocessor. On reflection, however, I concluded that although I was certainly interested in those topics I was not qualified to expound on them. Nevertheless it is a view I commend. What I am going to talk about is a subject which has occupied my spare time for the past ten years. The Journal.

I am grateful for the privilege of delivering the 14th Thomas Pullar Memorial Address.

The growth and development of an organisation is never purely circumstantial although the original formation is invariably in response to a need. This is certainly so in the case of the NZIMLT. It is commonly assumed that these things just happen but progress is always the result of perspicacious individuals seeing the need and seeking the solution. Such advances in our own organisation is looking after our own education and conducting our own negotiations which are now taken for granted, were the outcome of protracted activity usually against considerable opposition. We have been fortunate in having the right people at the right time. This was certainly so with the Journal, the brainchild of Douglas Whillans who also brought this cerebral infant to maturity. Indeed it would be more correct to declare that like Aphrodite, the Journal sprang fully formed from the wine-dark sea of his fertile imagination — if you will excuse this poetic lapse. (I had resolved to break it down and keep it simple.) Suffice to say that the Journal was started in 1946 as a quarterly publication and was not only edited but printed and published by Douglas Whillans. This project was most demanding in time and energy and eventually arrangements were made to have it printed commercially and published three times a year.

The next incumbent was Mr A. H. Murphy also of Auckland who carried the burden of Editorship for a further five years from 1951 to 1956. The publishing centre was then transferred to Christchurch when Mr J. J. Cannon assumed the duties in 1957. He experienced great difficulty in obtaining material for the Journal and resigned the position after this year. I could understand and sympathise with him. For the next four years, two editors, Miss L. Evans and Mr G. Rose, carried out the editorial function in tandem. The 1962 AGM agreed that Dunedin should now have a turn, and the Dunedin Branch appointed Mr J. Case as Editor. His period of office was marked by a number of innovations. The Newsletter was added and published six times a year, the octavo size was increased to quarto and the five-yearly term of office extended till his resignation in 1970 when he accepted a post in Australia. He had then

completed seven years in office. Because of the difficulties and complications involved in transferring the increasingly complex operation of publishing and advertising, the Journal remains in Dunedin.

It may be of some interest to recount some of the changes which have taken place during my term of office.

In 1971, the editor also looked after the membership listing which was embodied in a set of addressograph plates redolent of the Victorian era. In this day of computer lists I should perhaps explain that these were metal plates on which names and addresses were stamped letter by letter. The machine was a cross between a small pile driver and a plumber's nightmare. The plates were then fed into another infernal machine which imparted the information onto wrappers presented one by one. A pleasant afternoon could be whiled away in this idyllic fashion. The system was to gift-wrap each Journal individually after anointing the wrapper with glue. Volunteers had to be detailed for this operation. All going well, one-third of an old-fashioned ton of wrapped Journals embracing Newsletters and assorted trash, accumulated by late afternoon. It was my custom to load these onto a trolley and descend by lift to the post office. I recall on one occasion my arrival coincided with the arrival of the post office van. I approached, hot and grimy with glue up to the elbows, pushing my heavily laden trolley. The driver, a man of rare discernment, sized me up in an instant! "Right," he said, "Bring them out here," and kindly opened the door for me. I pushed the trolley out into the middle of the street, he opened the rear doors and I loaded the van under his direction. Well, I thought "Why not? Now I've done the bloody lot!"

One of the innovations I had in mind was to use a computer listing for the membership file and for posting, and after some searching about, Syd Shepherd used his good offices to gain access to the Hamilton Medical Laboratory Computer. This arrangement still continues, albeit somewhat shakily. The system still calls for labelling and sorting but packing and posting is carried out by the printers.

Another arduous job which seemed to me to be far outside the call of duty, was looking after the advertising. This meant the whole process from seeking advertising, defining copy requirements, arranging contracts, deadlines and terms, discussing type face and colour requirements, holding interminable conversations with advertising agents using a peculiar gobbledygook which seemed to be obligatory on those occasions and finally arranging the distribution of the advertisements in the Journal.

Lapsing into 19th century phraseology, one might say that, the editor had become such a repository of arcane knowledge that his demise would have occasioned a dreadful hiatus! This had to be changed. It was not done without trauma because it was necessary to seek another organisation willing to provide this service and to transfer the whole operation to them. Negotiations resulted in a satisfactory deal being reached with the Otago Daily Times (now Allied Press) in 1972. This arrangement has worked very well and continues to do so.

The editorial in the first issue of the Journal in April 1946, set out its aims quite clearly and simply. "It was the unanimous opinion of those present at the first annual general meeting of the Association, held in Wellington, that a Journal was a necessity as a means of keeping all the members of the Association acquainted with the progress of their fellow members, and the dissemination of all knowledge thought to be of interest and use." The editorial went on to remark, "The progress of the Journal and its value will however depend on the active support of all members."

On scanning these early pages one is immediately aware of another useful function of a Journal. It provides an historical record. Here for example is the "COP" examination for 1946 preoccupied with bacteriology. The salary scales starting at £130 and finishing at £600, and the complete membership, all eighty of them on one page.

There are other dimensions to a journal and one is to provide an identity for an organisation such as ours, which despite its not inconsiderable size, represents workers whose function is still largely unrecognised or whose work is rarely understood by the general public. It provides a means of recording the results of our labour and indicates the scope and diversity of our occupation. I can recall the opening of the Annual Conference in Wellington in 1971 when an address of welcome was given by Sir Francis Kitts then Mayor of Wellington and the opening ceremony was performed by Sir Arthur Porritt, then Governor-General. If one were to describe their meeting in the manner favoured by our forbears of the last century, one might say that both came armed with copies of the Journal and a friendly jousting ensued between these two worthy knights as to whose copy was most up to

date, in the course of which the protagonists brandished their furled journals at one another! Be that as it may, both referred to the Journal in their remarks and spoke knowledgeably of the history and aspirations of the Institute.

Journal publishing and printing is a complex and labour intensive operation and is consequently very expensive. The cost would be insupportable without the assistance of advertising revenue. I would like to record that most of the scientific suppliers in New Zealand provide continuing support for which we are grateful and our hope is that this happy symbiotic arrangement will carry on. Successful publications require a reliable financial base and a dependable source of material. The first requirement has generally been met in our case, but certainly not the second.

Many similar publications rely on examination-related theses or written components. We have unfortunately no such requirements in our present examination system and have to rely heavily on the goodwill and responsibility of our members. It is a matter for concern that although more and more work is undertaken, commented on, reviewed, modified and extended as shown by the deluge of papers presented at seminars and conferences, only a fraction of it finishes up permanently recorded in the Journal. I think that people are failing in their duties as technologists if they do not make the effort to present their work for publication.

I know that many people consider their work sufficiently new and important to merit publication in one of the specialist journals. This may be so, and indeed articles are often the product of several authors with different goals and allegiances, however there are two points I would make. The first is that our Journal is widely distributed throughout the world to subscribers, acquisition departments of universities and abstracting services. These have been detailed previously in annual reports.

The second point is that many articles are related to situations in New Zealand. Eleven such papers were read at the last conference. None of them were presented for publication. This causes me grave concern and mystification. Surely after the major effort of writing a paper has been accomplished it is simple enough to submit for possible publication.

When papers are received they are examined to see if they conform to the modest standards of rationality and literacy required. Quite often work has to be returned for alteration and sad to say, is never seen again.

Other folk refuse to submit their work on the ground that 'it is not good enough'. This preempts the editorial prerogative of pronouncing it 'not good enough' but there is also the implication that had the author the time, he or she could write something really quite splendid!

I started off with certain hopes and ambitions about the contents of the Journal and what its functions should be.

I wanted it to be useful in a practical sense. To be involved with quality control in every discipline and to publish results of regional and national surveys with suitable comments.

I wanted it to be educational in a broad sense but also to carry review articles of a basic nature for the benefit of student members. I wanted people to share their 'know-how' in technical communications.

I wanted correspondence and specialised columns. I thought the newly emerging disciplines would wish to promote themselves in our pages.

I had hoped most earnestly for a written component in our final examinations to provide motivation and material.

The last hope has never been realised, many other proposals and suggestions have been seed sown on stony ground but there have been some brief flowerings and a few hardy annuals.

Really the message is that the Journal is only as good as the contributions and the quality or lack of it reflects on the Institute members. In a sense, criticism of the Journal is self-criticism.

One other ambition I neglected to mention was to use larger type to improve legibility. A visit to the optician fixed that one.

The Newsletter started by my predecessor in 1969 as a separate project deserves mention. By being published six times a year it gave a better continuity in providing information on conditions of employment and other domestic affairs. Its increasing size poses an editorial problem but reflects our increasing involvement and responsibility for education and other matters. We have also reached the stage where we are able to look overseas and participate in the affairs of the International Association and these details are also published in the Newsletter.

I alluded to the increasing involvement of the Institute in education, conditions and salary negotiations. In every instance the NZIMLT has relied on voluntary effort to provide these major services. We have a very professional negotiating team; this is no area for amateurs. Institute

members play a major part on the Medical Technologists' Board which is largely concerned with education. The QTA examinations which in many respects are more complex administratively than the MTB examinations are organised by Institute members using a computer programme devised and written by an Institute member. There is an immense amount of work in all this.

The publications require daily involvement and as far as I know, all similar publications employ staff for the management function. Where there are separate Journals and Newsletters they are managed separately.

All this voluntary effort explains why our subscriptions remain at social club level. It may not always be feasible and it places a great deal of responsibility on the four per cent of members who are active.

Finally, I wish to comment on the role of the editor and his powers which are never too clearly defined.

The rules state that he shall be responsible to Council who shall determine his duties and powers. Council show a disinclination to do this and this is probably wise because a working relationship does evolve. The editor is *ex officio* a member of Council and is allowed to participate with the President's consent and I must confess sometimes without it. My view is that he also has a responsibility to the Institute members and should uphold the freedom of the press by disclosing all relevant information at his discretion and not act solely as a vehicle for Council's opinions. I am happy to say I do not have the problems encountered by the editors of some national newspapers and Councillors in the main restrict themselves to inviting the editor to publish choice items they have read somewhere, usually in the previous Newsletter.

This address is also my swansong as Editor.

I took on the task because it was a challenge which I thought I would enjoy and I wanted to make a contribution to the Institute. I thought a period of stability would be useful and that ten years was a nice round figure. Now my time is up.

It may be time for a new look or a fresh emphasis but whatever changes occur I hope you will give my successor every assistance next year.

There is a small postscript to all this. I have a message from the ubiquitous 'Fly-eye'.

You know that distant cousin of Spiderman and Superman who keeps an eye on everything at Conference?

Will he once more render an account of Conference in the September Newsletter?

Will he turn the spotlight on your performance, or yours, or even yours sir?

He wishes me to tell you he will!

And if he were here, he would now make his departure, straight through the roof at the speed of light, with a balloon coming out of his head saying PHROOM!!

Histology, Present and Future; A Review

B. C. Thackeray, FIMLS, ANZIMLT

Department of Histopathology, Waikato Hospital, Hamilton.

Received for publication, August 1980

Introduction

In 1970 Gardner[4] cast doubt on whether histopathology, 'already in the doldrums', would survive the next decade as an individual discipline. However, the past ten years has seen histopathology restored to its former place as a leader in medical laboratory science. The next decade has the potential of outstripping the great advances in pathological knowledge made during the second half of the 19th century.

Whilst the three fundamental techniques of the histologist — microscopy, microtomy and staining — have been retained, methodology incorporating a whole range of specialities has been applied to the diagnostic problems of the histopathologist.

The histopathologist continues to advise on the extent and spread of a malignant tumour, on the presence or absence of inflammation in an appendix, and on the cyclic changes present in the endometrium; but now has the power to aid the clinician when a disease process causes illness on a molecular level.

Today's Routine

The time honoured paraffin-processing of surgical and autopsy specimens may always have a place in the examination of pathological lesions which show changes at the cellular level. However, automation and mechanisation together with the development of sophisticated biopsying techniques have brought changes to the general routine and function of the department.

Pneumatic tube systems for transporting specimens from the operating theatre to the laboratory are functioning successfully in many new hos-

pitals. The pathologists macroscopic description is verbally recorded and stored in some form of memory bank for retrieval. Visual records are made with Polaroid film. Vacuum/heat assisted fixation/processing machines mean that as many as four 'surgical runs' may be performed in a day, radically changing the service provided by the hospital laboratory. The past decade has seen near perfection in knife sharpening machines. Labour saving embedding systems also contribute to specimen safety and simplify filing systems. A wide variety of staining machines are available, flexible enough to suit any laboratory routine. The on-line linear machines enable a 'haematoxylin and eosin' preparation to be available within 28 minutes of the section being cut. Some success has also been achieved with automatic coverslipping machines. The clerical side of the service has been streamlined through modern technology. Reports and records are handled using computer faculties providing the best method of acquisition, transmission, retrieval and storage of laboratory data. This has resulted in immense possibilities for retrospective research and increased the specificity and objectivity of pathological reporting. The essential features that a partly automated and mechanised system, for the routine and mundane work, have brought to the laboratory are:

1. Increased speed in test performance and report production.
2. Increased time to devote to work requiring manual skills and tests which are so infrequently requested that automation would be uneconomical.
3. Time to extend the range of routine techniques offered by the department by applying oneself to the new methods of histological investigation.
4. Time to investigate, develop and introduce new methods and techniques which will improve the quality and value of the service.

The latter features are worthy of deeper consideration particularly at a time when pathologists are starting to turn to the cytoplasm of cells for their answers in addition to the nucleus which has been regarded as diagnostically important for more than a century. The following section looks at some of the current techniques which augment or replace routine paraffin sections from which such information cannot be obtained.

Electron Microsopy

The advent of reliable and rapid embedding techniques means that electron micrographs can be produced in time to accompany conventional light microscopy preparations. Diagnosis, therefore, can be based on combined light and electron microscopy examination.

Although the collection of information about the ultrastructure of cells in health and disease has been a slow process, electron microscopy is becoming increasingly useful for solving diagnostic problems such as the identification of tumours whose histogenesis is uncertain by light microscopy, Carr *et al.* (1977).[1] It is essential in the examination of obscure pulmonary tumours as it enables both argyrophil granules and the characteristic inclusions of type II pneumonocytes to be identified, Kennedy (1977).[6]

Histochemistry

Histochemical techniques enable the identification and localisation of specific chemical substances in tissues. As such they are gradually replacing the empirical staining methods of the past. Enzyme histochemistry has advanced to a stage where it is comparatively simple to identify the activity of over one hundred enzymes in individual cells. While financial restrictions limit our access to electron microscopy the essential tools of the histochemist are the cryostat and a 37°C waterbath! Perhaps the most common example of enzyme histochemistry is in the diagnosis of muscle disorders which frequently rely upon the demonstration of ATP-ase and oxidative enzyme activity, Dubowitzy *et al.* (1973).[3]

Immunopathology

Specific substances may be identified in tissue if there is available a specific antiserum for that substance and some method of detecting the site where the specific antibody has been bound. This may be facilitated in histology by labelling the specific serum with a fluorescent dye (immuno-fluorescence methods) or with horseradish peroxidase (immunoperoxidase methods). The latter may be applied to paraffin embedded material as well as frozen sections enabling retrospective examination for the presence of immunoglobins. As well as specific immunoglobulins, immunoperoxidase methods may be used to detect cell products as an aid to diagnosis. Examples include carcinoembryonic antigen in colonic carcinoma cells, the trophoblastic elements of endodermal sinus

tumours which contain human chorionic gonadotropin, whereas the yolk sac elements contain alpha-fetoprotein. Cell products can also be a guide to prognosis. Breast carcinoma cells may contain β-1-glycoprotein and placental lactogen, whose detection may be used to assess prognosis, Horne *et al.* (1976)[5] using analogous methods. An area ready for considerable development is the demonstration of enzymes in paraffin sections by their antigenicity, instead of their ability to react with specific substrates, Reynolds (1980)[7].

Plastic Sections

The use of polymerising resins as embedding media for tissues has the great advantage over paraffin of producing much less distortion. In addition, because thinner sections may be cut greater clarity of cellular relationship and cytoplasmic detail is achieved often revealing further information. This has become a valuable adjunct to routine paraffin processing and because of the parallels in principle with routine processing requires little practice to master. Most of the commonly used tinctorial methods can be applied with little adaptation.

Quantification

Many histopathological opinions rest largely on the subjective impressions of the observer based upon previous experience. In many cases, these can be backed up by quantitative analysis. Simple examples are the measurement of the height of the intestinal villi in coeliac disease or the volume of the mucous glands in chronic bronchitis.

Many laboratories use television image analysis to measure the relative areas and thus volumes (Delesse's theorem) of substances in stained histological sections. This has wide applications, for example the quantities of osteoid and calcium in metabolic bone disease.

There is of course much room for overlap between these disciplines and much work has already been done in the application of electron microscopy to enzyme histochemistry and immunopathology for example.

The Future

So what of the future? Surely the day is not far off when we will have a medium not only suitable for embedding but which 'fixes' as it quickly infiltrates. In industry ultrasonics and laser beams are used to cut metals, plastics and ceramics; the combination of a resin block and an ultrasonic pencil could facilitate automatic sectioning. Histopathology has long been the two dimensional study of three dimensional specimens. The advent of the scanning electron microscope with magnifications as low as ×30 and the application of quantification methods long used in geology may re-write the current pathology texts. Automatic scanning light microscopy based on quantification of components of stained sections as a means of diagnosis is only a few steps away from the already successful methods mentioned under the general heading of quantification.

Conclusions

Although originally concerned exclusively with structure, histology also sheds light on function by providing information about the minute organisation of cells and tissues to supplement the sciences of physiology and biochemistry which deal with the nature and activity of the tissue components rather than their localisation, Disbrey *et al.* (1970)[2].

More than ever we can say that histological technique has become a science whilst still an art.

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A Case of Biclonal Paraproteinaemia

R. D. Allan

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Received for publication, April, 1980

Summary

A case of biclonal paraproteinaemia is described along with the laboratory methods used for its detection and characterisation. Initially an IgA lambda monoclonal gammopathy was detected accompanying a plasma cell myeloma.

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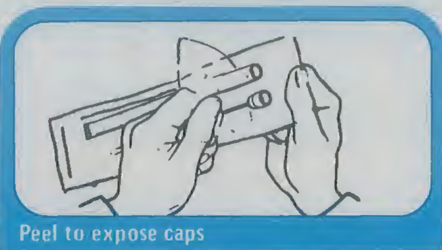
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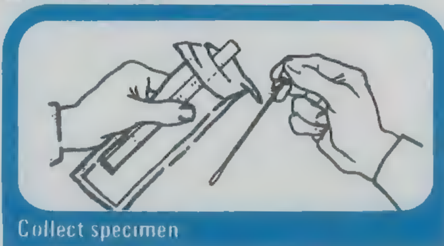
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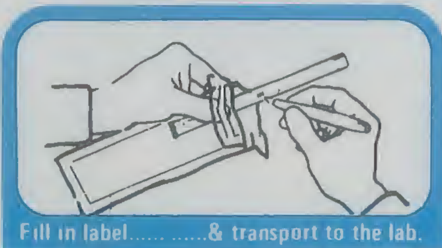
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Three years after the condition was discovered a further paraprotein developed which proved to be an IgG lambda paraprotein. The two discrete bands were obvious from the zone electrophoresis, the IgG was raised, immunoelectrophoresis disclosed abnormal IgA, IgG and lambda light chain fractions and immunofixation revealed two discrete lambda light chain fractions coincident with the IgA and IgG fractions.

Introduction

Multiband paraproteinaemia is not uncommon and 11% of a large group of patients with paraproteinaemia were reported to show multibands. Gore *et al.* (1979)[2]. Only 60% of the cases with multibands were associated with myelomata and a wide variety of conditions gave rise to multibands. The paraproteins numbered up to four and consisted of various combinations of the same type or different types, with and without light chains. IgA occurred most commonly and in myelomata had the worst prognosis.

Case History

The patient, born in 1940, had been attending the Rheumatology Clinic from about 1973, suffering from joint pains and tiredness. Previous history referred to the removal of a mole and she had a number of pigmented moles. A raised ESR and proteinuria were investigated and further tests revealed an IgA monoclonal gammopathy with an associated plasma cell myeloma. As she was pregnant she was treated conservatively and had a healthy child. Over the next three years the number of plasma cells in the marrow increased as did the IgA paraprotein and the ESR continued to rise. The figures quoted are 8-20% plasma cells. ESR 70-120 mm and the IgA 20-30 g/litre. The IgG and lgM remained around 5 g/litre and 0.6 g/litre during this period from 1975-78. Other haematological and biochemical parameters were essentially normal including calcium and albumen. Total protein remained around 85 g, just above our reference range.

In May 1979 two discrete bands were noted in the zone electrophoresis and the IgG level had jumped up to 15 g/litre. The IgA remained around 30 g/litre and the IgM was 0.3 g/litre. The ESR had risen to 136 mm.

Chemotherapy was started in August 1979 and has been continued with some modifications up to the present. The current blood parameters are ESR 63 mm, IgA about 30 g/litre, IgG 11.5 g/litre and IgM 0.4 g/litre.

The patient remains generally well.

Methods

Cellulose acetate strips stained with ponceau S were used for zone electrophoresis using Helena apparatus and methodology. The strips were scanned with a 'Quick' Densitometer produced by the Atago Optical Works Co. Ltd, Japan. There was nothing unusual about the technique or apparatus.

Quantitation of the immunoglobulins was done by single radial immunodiffusion with commercial plates using the Fahey Technique. Immunoelectrophoresis was performed on slides covered with 1% agarose in 0.02μ Owen's Barbitone Buffer. This was diluted from a 0.1μ solution consisting of 20.0 g sodium barbitone, 7.84 g anhydrous sodium acetate, 136.8 ml of 0.1 M HCl, H_2O up to 2 litre.

A Shandon slide-holder was used to hold the slides and the troughs and wells were stamped out with a Shandon Cutter. The same buffer was used in the electrophoretic bath. Constant current of 2.5 ma/slide was used and this gave about 60v. The tests were run in parallel with normal controls for comparison. The normal control material was tinted with bromocresol green to indicate the distance the albumen had run. Time usually about 1 h. About 1μl of serum sample was placed in the wells. When electrophoresis was finished, the intervening troughs were removed from the agarose with a square cut needle attached to suction, and 70μl of the specific antisera added. The slides were left in a moist atmosphere overnight, to develop. The fluid was pressed out of the agarose by covering the slides with two sheets of Whatman No. 1 filter paper and several thicknesses of paper towels surmounted by a piece of plate glass weighing about 2 kg. After at least 15 m the slides were gently washed in buffered saline for an hour. The buffer consisted of 8.5 g Nacl, 8.62 g Na_2HPO_4 $2H_2O$ and 2.48 g KH_2PO_4 in a litre of water.

The slides were then repressed and dried with a hair-dryer till transparent. Finally they were stained with Comassie Blue. The technique of pressing and staining was described originally by Laurell (1972)[4] and appears in the Behring literature. (Methods of Qualitative and Quantitative Immunoelectrophoresis.)

Immunofixation was described by Alper *et al.* (1969)[1]. They succinctly described it as a technique "which allows the worker to anchor *in*

situ, protein of a single immunologic species by exposing the protein containing gel to specific antibody after electrophoresis". The practical application employed in this instance generally follows the methods described by Ritchie *et al.* (1976)[3].

Zone electrophoresis was carried out on agarose gel using the Hoechst electrophoresis apparatus with a water-cooled bridge and gel-frame. Glass plates 205 x 110 x 1.5 mm were generally used but sometimes a smaller size for single specimens. 1% agarose (L'Industrie Biologique Francaise) in a TRIS buffer was used. (TRIS 23 g, barbituric acid 15 g, sodium barbitone 47.5 g, calcium lactate 2 g, H_2O to 5 litre.) This buffer was also used for the electrophoretic bath. About 30 ml of agarose at approximately 80°C is poured onto the large glass plate on a levelling table. A metal comb or gel-puncher is set in the molten agarose so that a layer of agar remains under each indentation. After a few minutes when the agarose has set the punch is removed and excess buffer removed with a sharply cut piece of filter paper. 3-5μl of serum is pipetted into the well. A control serum with bromocresol green acts as a marker during electrophoresis. The plate is placed on the bridge and contact made with the gel frame with strips of "XLO" cloth. (Used for cleaning purposes.) The output from the rectifier is adjusted to 15v/cm and electrophoresis is allowed to proceed until the albumen marker has travelled 3.2 cm from the point of application. This takes about 1 hour. It is necessary to distinguish between excess dye which moves ahead, and albumen.

Specific antiserum is applied to a piece of cellulose acetate 3 x 1 cm. About 70μl is required to soak it to a transparent state. This is carefully placed on the appropriate portion of the agarose to cover the gamma region fore and aft of the application and reaching to the alpha$_1$ region. Leave in the moist atmosphere of the bath for 1 hour.

Pressing, washing and repressing and staining with Commassie Blue is carried out as described for the immunoelectrophoresis method.

Results and Discussion

The sequence of events in this particular case was determined by the need to characterise the biclonal paraproteins disclosed by the zone electrophoresis (Figures 1 and 2).

These illustrate the original monoclonal gammopathy and the emergence of the second band.

The abnormal IgG, IgA and lambda light chain

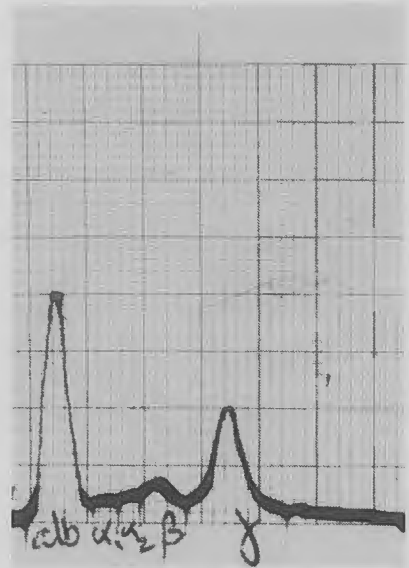

Figure 1.—1975. Zone electrophoresis scan showing single monoclonal band.

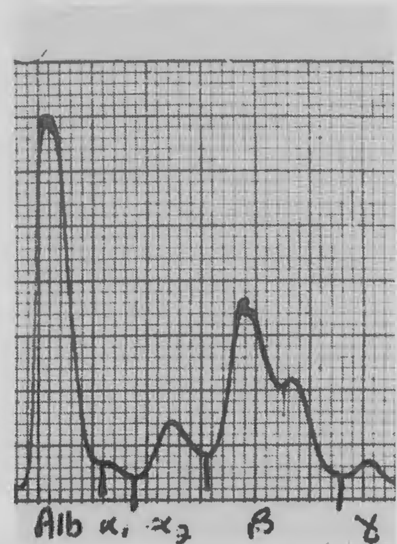

Figure 2.—1979. Zone electrophoresis scan showing two monoclonal bands.

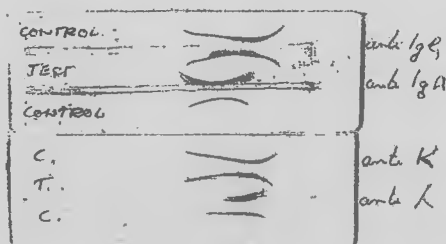

Figure 3.—Immunoelectrophoresis showing abnormal IgG, IgA and lambda arcs.

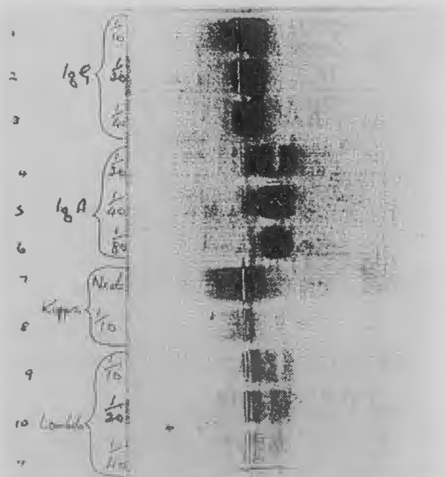

Figure 4.—Immunofixation preparation described in text.

are shown in the illustration of the immunoelectrophoresis slides (Figure 3).

Immunofixation was used at this point to see if the IgG paraprotein could be further characterised and the result is illustrated (Figure 4).

Several points are worth mentioning. Dilutions of the patient's serum were employed so that optimal precipitation could be achieved. An excess of antigen has a soluble effect and shows up as an etched area. This can be seen in the case of the IgA 1/20 dilution and in the lambda 1/10 dilution. It seems that a protein concentration of less than 1 g/litre is needed for optimal precipitation.

It can be seen that two separate lambda light chain discrete bands have developed *in situ* corresponding to a discrete IgG and a discrete IgA band indicating that both immunoglobulins had lambda light chains. The immunoelectrophoresis preparation would usually depict these as a continuum so preventing differentiation.

Acknowledgment

I wish to thank Dr L. A. Bates, Clinical Haematologist, Otago Hospital Board, for providing clinical details and for permission to use the material to illustrate the techniques used.

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The Assessment of Fetal Lung Maturation using Amniotic Fluid Palmitic Acid Quantitation

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Introduction

The risk of a fetus developing respiratory distress syndrome is considered to be minimal when the lecithin to sphingomyelin ratio (L/S) is greater than 2 to 1. Gluck, *et al.* (1971)[3]. Further work has revealed that the dipalmitoyl species form the major component of the total lecithin fatty acids during the last trimester, in amniotic fluid, Lindbach (1976)[6]. Warren *et al.*(1973)[8], (1979)[9], described a method for quantitating amniotic fluid palmitic acid using gas chromatography. This involves a number of extraction procedures and is time-consuming.

In this communication, a rapid technique for quantitation of amniotic fluid palmitic acid using gas chromatography based on the methylation

procedure of Morrison and Smith (1964)[7] and Bichler *et ali* (1977)[1] is assessed for specificity and reliability in relation to predicting fetal lung maturation.

Materials and Methods

Reagents

Boron-trifluoride methanol complex (14% BF_3) was obtained from either Analabs, McCaw Ethicals Ltd., Auckland, or British Drug Houses, Poole, Dorset, England. The reagent from both manufacturers gave satisfactory results. Lecithin, fatty acids and fatty acid methyl esters were all obtained from the Sigma Chemical Co., St. Louis, U.S.A. The remaining reagents were all of Analar grade, no further purification of solvents was undertaken.

Preparation of Standards

A composite standard containing one mmol/litre of decanoic, lauric, myristic, heptadecanoic, stearic, oleic and linoleic methyl ester fatty acids was prepared in chloroform. This standard serves as a reference when identifying peaks other than palmitic acid. An additional standard containing 1.5 mmol/litre palmitic acid and heptadecanoic methyl ester fatty acids were also prepared in chloroform; this was used as a calibrating standard. An internal standard containing 2 mmol/litre heptadecanoic acid methyl ester in chloroform was also prepared.

Samples

Amniotic fluid was collected by transabdominal amniocentesis for fetal maturity assessment and centrifuged immediately after collection. All samples had an L/S ratio performed and quantitated as described by Legge (1976)[5]. If the palmitic acid assay was to be done at a later date, the sample was frozen at -20°C. Samples contaminated with blood or meconium were not used.

Method

The lipids were extracted from the amniotic fluid according to Folch *et al.* (1957)[2]. To 2 ml of centrifuged amniotic fluid, 4 ml of chloroform and 2 ml of methanol was added. The mixture is shaken vigorously for 5 minutes. It is then centrifuged for 10 minutes at 2,500 rpm; the upper aqueous phase is removed and discarded. The lower organic phase is filtered through a Whatman I.P.S. filter paper.

Three ml of the filtrate is pipetted into a small quick-fit pear-shaped flask, 0.2 ml of internal standard is added and evaporated at 50°C using either a vacuum or a stream of dry nitrogen. To the residue 5 ml of boron-trifluoride-methanol reagent is added and the flask is shaken to dissolve the residue. The flask is sealed containing a nitrogen atmosphere and heated at 85°C for 15 minutes. At the end of this time the seal of the flask is removed and the flask and its contents are cooled in a stream of running water. The methylated fatty acids are extracted by adding two volumes of n-Hexane and one volume of distilled water and shaking the mixture for two minutes. The mixture is then centrifuged at 2,500 rpm for five minutes. The n-Hexane layer is removed and a few mg of anhydrous sodium sulphate is added to remove any water contamination. The n-Hexane layer, 2-4 μl, is then ready for injection into the gas chromatograph.

Gas Chromatograph Conditions

A Philips G.C.D. gas chromatograph with flame ionisation detectors and five foot glass columns filled with 10% diethyleneglycol succinate (DEGS) on acid washed and sialonised Chromosorb W. (80-100 mesh) is used. The gas flow rates were hydrogen and nitrogen 40 ml/minute, air 600 ml/minute. For fatty acid analysis C-10 (decanoic) to C-20 (arachidic) a temperature programme was used over the range of 120-195°C at a rate of 4°C per minute. For palmitic acid analysis only, the assay is performed using isothermal conditions operating at 175°C. A detector temperature of 200°C and injection temperature of 220°C was used.

Quantitation

Quantitation of the fatty acids was based on the technique of Labadie *et al.* (1974)[4] using a Spectrophysics Acculab computing integrator.

Clinical Outcome

One hundred third trimester amniotic fluid specimens for L/S ratios were compared with the palmitic acid concentration. The neonatal outcome of all infants was examined by searching medical records to determine whether those delivered with a 'mature' palmitic acid developed respiratory distress.

Results

Typical chromatograms of amniotic fluid fatty acid methyl esters from a fetus with 'immature' lung function and a fetus with "mature" lung function are shown in Figures 1 and 2 respectively. The concentration of amniotic fluid

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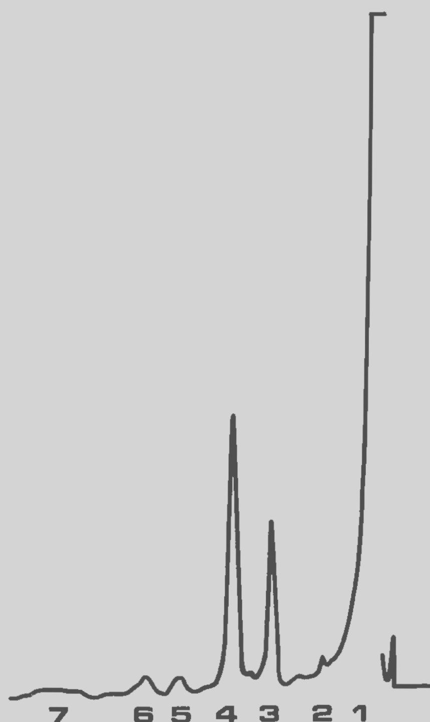

Figure 1.—Gas chromatograph tracing of fatty acids in amniotic fluid at 33 weeks gestation. Peaks are: 1. solvent, 2. myristic acid, 3. palmitic acid, 4. heptadecanoic acid (internal standard), 5. stearic acid, 6. oleic acid, 7. linoleic acid.

Figure 2.—Gas chromatograph tracing of fatty acids in amniotic fluid at 38 weeks gestation. Peaks are: 1. solvent, 2. myristic acid, 3. palmitic acid, 4. heptadecanoic acid (internal standard), 5. stearic acid, 6. oleic acid, 7. linoleic acid.

palmitic acid increased as the fetal lungs matured. This agrees with the findings of Bichler *et al.* (1977)[1]

The correlation between LSAR (y axis) and palmitic acid concentration (x axis) is shown in Figure 3 with the standard error of estimate of y on x indicated by the broken line. A linear correlation exists between the two assays (correlation coefficient 0.956). The equation for the regression line was $y = 0.749 + 10.847x$. Using this equation the palmitic acid concentration can be calculated to be 0.153 mmol/litre when the LSAR is 2.4: the criterion used in the author's laboratory for indicating fetal lung maturation.

All infants who were delivered with amniotic fluid palmitic acid concentrations of 0.135 mmol/litre or greater did not develop respiratory distress syndrome. This level corresponds to a LSAR of 2.2 and represents a base level below which fetal lungs would be considered to be 'immature'.

Discussion

The method described differs from other amniotic fluid palmitic acid assays in that it uses a direct methylation procedure. The method takes less than one hour to complete and gives good separation of fatty acid methylesters. A similar technique has been described by Bichler *et al.* (1977)[1]. This technique was investigated but it was found that the small (1 ml) amniotic fluid sample and the lower (60°C) methylation temperature gave variable results in the author's laboratory.

As most of the palmitic acid in amniotic fluid originates from lecithin, Warren *et al.* (1973)[8], (1979)[9], a good correlation would be expected between the L/S ratio and palmitic acid concentration. This was found to be true throughout the range of concentrations, indicating the specificity of the technique. The palmitic acid level also correlates well with fetal outcome in that no infant delivered with a 'mature' amniotic fluid palmitic acid concentration has developed respiratory distress

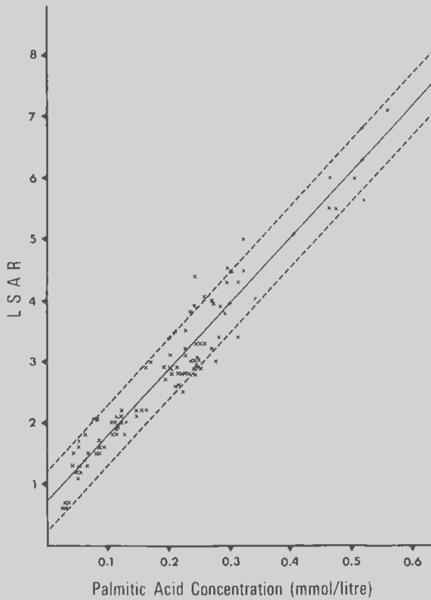

Figure 3.—Comparison of LSAR and palmitic acid concentrations in 100 amniotic fluid samples.

__________ = regression line
_ _ _ _ _ = standard error of estimate of y on x.

syndrome. Fetal lung maturation would be indicated if the palmitic acid concentration was 0.135 mmol/litre or greater. This level is considered to effectively separate 'immature' and 'mature' fetal lung function.

The significance of the fatty acid patterns is currently being investigated.

Acknowledgment

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Metronidazole Interference with Continuous Flow Measurement of Aspartate Aminotransferase

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Summary

This paper reports the factitious depression of patients' aspartate aminotransferase (AST) activity by metronidazole therapy. False low results are observed when AST is measured by continuous flow spectrophotometry. Attempts to eliminate this interference by incorporating a blank channel in the manifold are described.

Introduction

Aspartate aminotransferase is offered by this laboratory as part of liver function and cardiac enzyme screens. In late 1978 the laboratory replaced Technicon AutoAnalyzer I equipment with the Chemlab microprocessor-controlled continuous flow system. Simultaneously the AST methodology was updated. The Fast Ponceau L coupled procedure (Morgenstern *et al.*, 1966)[3] was replaced by a malate dehydrogenase/reduced nicotinamide adenine dinucleotide (MDH/NADH) linked method. During routine use of the Chemlab abnormally low and even 'negative' AST results were occasionally observed. The frequency of these observations increased and in September 1979 investigation of their cause was initiated.

Materials and Methods

Sera were from patients' samples submitted to the laboratory for routine liver function tests. Flagyl (Metronidazole B.P., 5 g/litre, May & Baker N.Z. Ltd) was obtained from the hospital

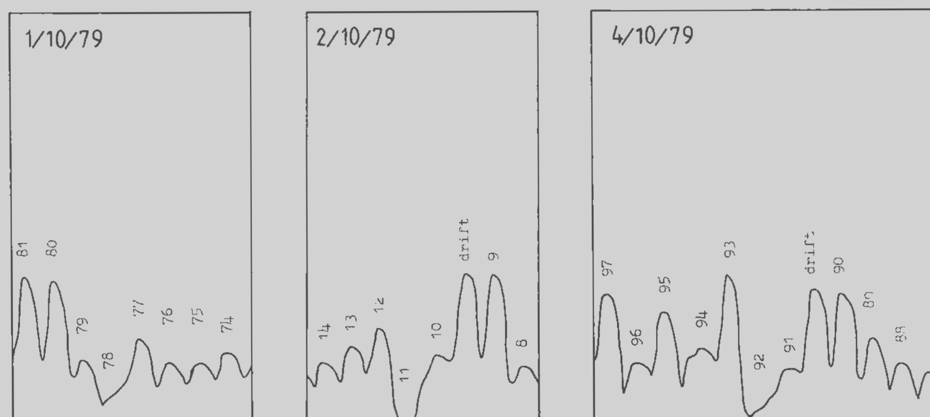
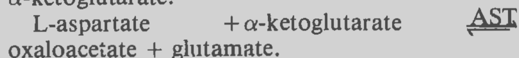


Figure 1.—Routine Chemlab charts showing interference on AST peaks. For clarity, the alkaline phosphatase peaks have been deleted.

pharmacy. MDH/NADH vials, L-aspartic acid and α-ketoglutaric acid were purchased from Sigma.

Routine and experimental AST estimations were performed on the Chemlab using the Technicon AAII-10 test method. A Gilford 300 N colorimeter, with a flow cell modification, was coupled to the Chemlab because the original Chemlab colorimeters cannot transmit light at 340 nm (Wilson and Chilwell, 1980)[8]. The system was calibrated with Versatol Automated Hi reference serum (General Diagnostics) and drift was monitored with an in-house bovine pool (Parker, 1980)[4]. Experimental AST estimations were also done at 30°C on the IL Multistat III (Instrumentation Laboratory) using Dynazyme II AST reagent (Baker Diagnostics).

The coupled assay system consists of two reactions: in the first AST catalyzes the transfer of an amino group from aspartate to α-ketoglutarate:



L-aspartate + α-ketoglutarate $\overset{AST}{\rightleftharpoons}$ oxaloacetate + glutamate.

In the presence of malate dehydrogenase and NADH the second reaction proceeds:

oxaloacetate + NADH + $H^+ \overset{MDH}{\rightleftharpoons}$ malate + NAD^+.

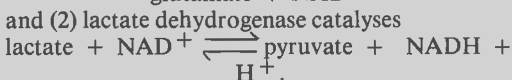
The disappearance of NADH is measured at 340 nm and is proportional to the AST activity. In continuous flow systems serum samples and substrates are mixed and incubated at 37°C. The product of the first reaction, oxaloacetate, is dialyzed into the recipient MDH/NADH stream and the decrease in absorbance relative to baseline is displayed as peaks on a chart recorder.

Results and Discussion

Typical examples of routine Chemlab charts are given in Figure 1. Specimens 78, 11, and 92 (individual charts from left to right) show abnormal peak profiles; in fact they were recorded by the data processor as 'negative' AST results and flagged as error peaks. These negative AST peaks are equivalent to an apparent production of NADH rather than its utilisation. Sample derived enzymatic interferences (Bergmeyer, *et al.*, 1977)[1] are eliminated by the presence of the dialyzer in the manifold, that is, only low molecular weight compounds can enter the MDH/NADH stream. Conceivably, similar interfering reactions could arise from enzyme contaminants in the MDH preparation for example (1) glutamate dehydrogenase catalyses

α-ketoglutarate + NADH + $NH_4^+ \rightleftharpoons$ glutamate + NAD^+

and (2) lactate dehydrogenase catalyses



lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH + H^+.

The MDH/NADH reagent was not tested for the presence of glutamate dehydrogenase. However, in the unlikely event that the concentration of NH_4^+ was sufficient for (1) to proceed, NADH would be consumed resulting in falsely *raised* AST. The potential interference of elevated serum lactate was eliminated when it was found that the MDH/NADH reagent was devoid of lactate dehydrogenase activity (Multistat III, IL reagent). Furthermore, the assay conditions (pH 7.4) would tend to favour the reverse reaction.

It seemed likely, therefore, that a low molecular weight compound was dialyzing from serum into

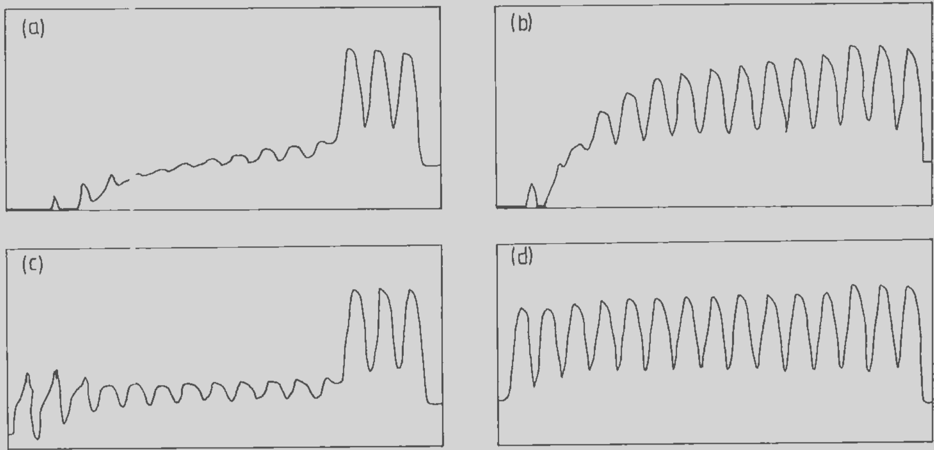


Figure 2.—Effect of increasing metronidazole concentration on AST peaks. Charts are read from right to left. Each experiment was started with three samples of bovine pool followed by serum samples containing metronidazole at 0, 1, 2.5, 5, 7.5, 10, 15, 20, 30, 50, 75, and 100 mg/litre.

(a) normal AST samples without blank channel
(b) raised AST sample without blank channel
(c) normal AST sample with blank channel
(d) raised AST sample with blank channel

the recipient stream giving an increase in absorbance at 340 nm. Investigation of the patients' drug history revealed that Flagyl (metronidazole) was the common therapy. Metronidazole, (2-methyl-5-nitroimidazole-1-ethanol) is an antibiotic effective against obligate anaerobes and is commonly used in the prevention of post-operative infection. Metronidazole has an absorbance maximum at 320 nm but 60% remains at 340 nm. A 25 mg/litre solution of metronidazole in phosphate buffer, pH 7.4 has an absorbance of 0.73 at 340 nm (Unicam SP 800 10 mm light path).

The effect of increasing amounts of metronidazole on normal and raised AST levels was tested and the results are shown in Figure 2. Normal AST values were markedly depressed at low drug levels and at concentrations of greater than 10 mg/litre of serum 'negative' AST values resulted. Plasma concentrations of metronidazole have been reported in the range of 6.8-47.5 mg/litre after intravenous therapy (Wheeler *et al.*, 1978)[7]. It was confirmed that the interference was entirely optical since the drug had no effect on AST estimation when the samples were assayed kinetically, on the centrifugal analyzer (Table I).

TABLE I

Kinetically determined AST activity in the presence of metronidazole

Metronidazole concentration (mg/litre)	Normal AST sample (I.U./litre)	Raised AST sample (I.U./litre)
0	20	50
1	18	50
2.5	19	52
5	20	50
7.5	21	50
10	20	52
15	20	49
20	19	50
30	20	50
50	20	50
75	21	50
100	21	51

AST activities were assayed at 30°C on the IL Multistat III.

It was hoped to overcome the problem by installing a serum blank channel in the manifold, since laboratory organisation did not permit routine AST estimations on the Multistat III. The Gilford 300 N was replaced with a Technicon

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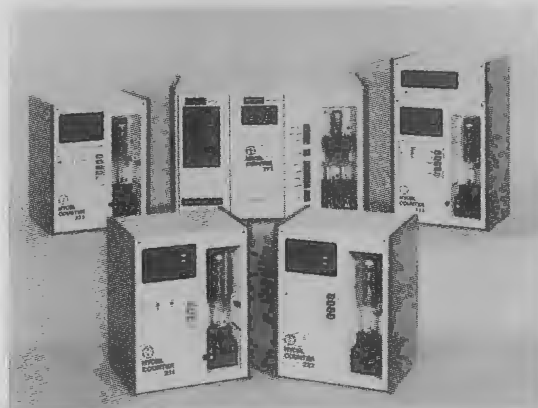

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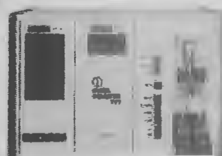

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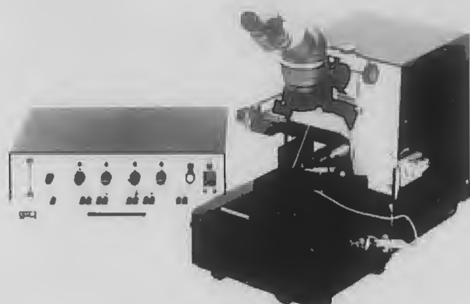

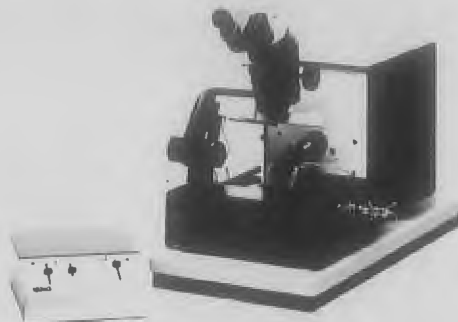

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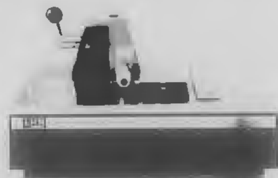

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AutoAnalyzer single-channel 340 nm colorimeter. The latter being interfaced without difficulty, into the Chemlab data processor. The modification was not entirely successful in eliminating the interference (Figure 2). Peak heights decreased slightly with increasing metronidazole concentrations and peak profiles were aberrant at high drug levels; the latter being emphasised at normal AST activities. Possible reasons for these observations are: (1) imprecise phasing of the blank and analytical reagent streams; and (2) spectrophotometric insufficiency of the colorimeter. Phasing of the manifold was checked visually using recorder ink in the reagent streams. Coombs and Rabin (1974)[2] have reported interference of NADH absorbance by Flagyl and they demonstrated, by difference spectrophotometry, that this was caused by failure of the spectrophotometer to measure the true absorbance of NADH in the presence of a high background of Flagyl absorbance. In their work the effect was attenuated by replacing 10 mm cuvettes with 2 mm cuvettes. The Technicon colorimeter uses a rather long 15 mm pathlength flow-cell and baseline absorbances of the reagent streams are 0.7-0.9. Addition of high concentrations of metronidazole (serum samples greater than 30 mg/litre) further increases baseline absorbance — approaching the limits of the colorimeter and when the A340nm difference between channels is small (i.e. low AST activities) noisy peaks result. Furthermore, any slight phasing imprecision would be amplified under these conditions.

A number of drugs known to interfere with AST methodology are listed by Young *et al.*, (1975)[9]; Flagyl is not amongst them. Whilst this investigation was in progress, Tighe and Jones (1979)[6] reported similar findings to those described in this present paper. Metronidazole at concentrations of greater than 15 mg/litre gave negative results for a sample with normal AST activity. Subsequently, a literature search revealed that this metronidazole effect has been documented previously (Rissing *et al.*, 1978)[5].

The modified AST manifold has been in routine use for one month. Despite its limitations, negative and abnormal AST peak profiles appear to have been eliminated.

Acknowledgments

The authors thank Mr Ron Zoest, Sci-Med (N.Z.) Ltd, Dunedin, for interfacing the Technicon colorimeter with the Chemlab data processor. Comments from, and discussions with the staff of the laboratory were appreciated.

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A Preliminary Evaluation of a Micro-method for Erythrocyte Sedimentation Rate

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Introduction

The Erythrocyte Sedimentation Rate (ESR) although a non-specific test is used universally by clinicians as a diagnostic tool as an index of the progress of certain chronic disorders.

The "standardised selected method" as recommended by the International Committee for Standardisation in Haematology (ICSH) (1977)[3], is the Westergren method although modifications or alternative techniques may be accepted as long as their validity is established with this reference method. There is often a need for estimation of the ESR in patients where insufficient specimen is available for an ESR by the recommended method. In these cases it is not always possible nor desirable to obtain large amounts of blood from neonates and young children. The application of the ESR to a micro-method has not been well documented since the first method by Landau. (1933)[4].

The purpose of this study was to initiate a preliminary trial of a micro-method Guest and its feasibility for routine use. The Guest micro-method has the following advantages 1. Can measure a micro ESR as only a small volume of blood required. 2. Eliminates risk of transmitted disease, particularly hepatitis, by at all times avoiding direct contact with the blood sample. Adler *et al.* (1975)[1], Evans *et al.* (1970)[2], Niejadlik *et al.* (1977)[4].

Materials and Method

The Micro ESR Kit (Figures 1 and 2) contains:

1. Dispette stand which holds ten Dispettes, and has a slide out tray so that blood and tubes are removed without handling after reading. An added feature is the incorporation of a levelling bubble so that it can be adjusted to stand perfectly level.
2. 200 mm Dispette blood column with a 1 mm diameter bore graduated in mm from 0 - 150 and plugged with cotton wool at the top to the '0' mark.
3. Hand Pump (electric or manual).

The method required the addition of 4 parts fresh whole blood to 1 part 3.8 percent trisodium citrate and this solution MUST be mixed and placed in the Dispette stand.

A plugged Dispette is dropped through the allocated holes in the stand ensuring that the plug/zero is at the top, and base of the Dispette rests in the bottom of the tube container.

The pump is pressed onto the top of the plugged Dispette and blood is quickly aspirated to the level of the plug. This ensures the blood is level at the '0' mark. After 1 h duration, the level of blood is read and noted.

After reading, the stand is picked up, the slide tray removed so that the Dispettes and blood containers fall into the waste bin without manual contact. The slide tray can then be replaced in the rack.

Evaluation

The evaluation was carried out in the following manner:

1. Correlation with the modified Westergren Method.
2. Suitability for Micro collect and use.

The ICSH recommend that alternative techniques should be correlated with the standarised method. The Guest Dispette Macro-method currently in routine use in most hospitals has already been compared, and so correlation of

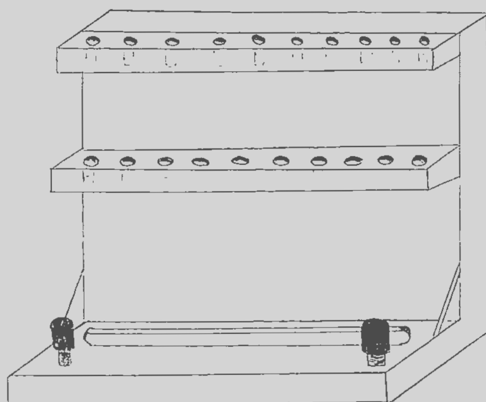

Figure 1. Dispette stand.

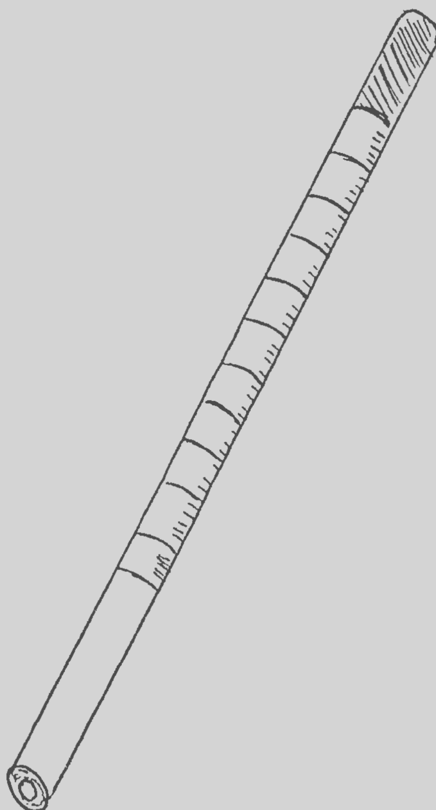

Figure 2. Dispette blood column.

the new Micro-method with this method was carried out.

Thirty bloods were selected and two dilutions containing 2 ml fresh whole blood and 0.5 ml 3.8 percent trisodium citrate were made, one dilution being used to determine the ESR by the modified Westergren method (Guest) and the other by the Micro-method as described above.

The ESRs on both methods were read at one hour to the nearest millimetre. See Table I. These results confirmed that there was an acceptable correlation between the two methods.

Table I

Sedimentation Rates

Sample	*Westergren Method*	*Guest Micro Method*
1	2	2
2	1	1
3	2	1
4	7	7
5	5	6
6	3	4
7	4	3
8	3	3
9	8	8
10	11	12
11	19	22
12	18	20
13	22	24
14	12	15
15	45	47
16	33	33
17	30	34
18	16	15
19	48	45
20	63	65
21	81	85
22	37	35
23	23	28
24	132	129
25	55	60
26	42	45
27	89	92
28	90	90
29	44	44
30	97	105
Mean	34.7	36.0

The next step was to establish the smallest critical volume of blood required to give valid ESRs by this method. This was done by setting up a series of dilutions keeping the ratio of 4:1 blood and 3.8 percent trisodium citrate constant. Each ESR dilution was determined by the Micro Guest ESR system. Dilutions were made in 2 ml auto-analyser cups, which fitted easily onto the slide out tray provided.

Dilution series

(1) 2 ml Blood 0.5 ml 3.8% trisodium citrate
(2) 1 ml Blood 0.25 ml 3.8% trisodium citrate
(3) 0.8 ml Blood 0.2 ml 3.8% trisodium citrate
(4) 0.4 ml Blood 0.1 ml 3.8% trisodium citrate
(5) 0.2 ml Blood 0.05 ml 3.8% trisodium citrate

Table II

Results

Sedimentation rates

	2 ml Blood	*1 ml Blood*	*0.8 ml Blood*	*0.4 ml Blood*	*0.2 ml Blood*
(1)	10	10	8	10	10
(2)	26	27	28	28	30
(3)	120	120	115	118	118
(4)	52	53	50	53	52
(5)	5	5	7	7	5
(6)	25	25	24	24	24
(7)	96	100	102	101	100
(8)	69	69	68	64	65
(9)	33	31	33	35	31
(10)	43	43	47	50	47

This preliminary trial suggests that a dilution of 0.2 ml blood mixed in 0.05 ml 3.8 percent trisodium citrate gives comparable results to the modified Westergren method. It is possible for 0.3 ml of blood to be obtained from neonates, small children and difficult patients by the BD Microtainers No. 5961. This formerly contained 0.23 mg EDTA per reservoir. In many instances this amount was insufficient to prevent clotting.

The amount of EDTA in the BD microtainers No. 5961 has now been increased to 0.3 mg.

Suggestions for using this Micro ESR method:

1. 0.3 ml blood is collected under "standard" micro conditions into the microtainer tube. (See B.D. Microtainer package insert.)
2. 0.2 ml removed by automatic pipette and mixed in 0.05 ml 3.8 percent trisodium citrate.
3. ESR determined by micro-method described earlier.

Conclusion

This preliminary evaluation shows a good correlation between a micro method and the routinely established Guest Dispette Method. It shows accurate reproducible results with small volumes of blood allowing ESR determination in the neonate or difficult collect. It has the added feature of no manual contact with the blood sample eliminating risk of infection.

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ABO Haemolytic Disease of the Newborn; A New Plymouth Study

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Summary

In Haemolytic Disease of the Newborn (HDN) due to ABO incompatibility the serological findings are much less helpful in establishing the diagnosis than in HDN due to anti Rh. Mollinson (1972)[3]. A study of infants born during a four year period in the maternity wards at Taranaki Base Hospital and at the Opunake and Waitara Maternity Annexes suggests that the incidence of ABO HDN is low.

Introduction

ABO HDN since first described by Halbrecht (1944)[2] has been the subject of many investigations and attempts to predict its occurrence. Unfortunately methods using 2 mercaptoethanol, dithyothreitol, polyvinylpyrolodine, erythrocyte sedimentation rate, Direct Coombs Tests using various modifications and many others devised to date, suffer from one common disadvantage; they detect foetal-maternal ABO incompatibility and not clinical HDN. The only reasonably reliable method of predicting the disease is that of the mother having had a past history of an ABO incompatible infant with severe hyperbilirubinaemia.

Although it is well known that high levels of bilirubin in neonates can cause kernicterus a number of papers in recent years have shown that it is not only the level of bilirubin in the serum but the length of exposure to high serum levels of bilirubin that can be responsible for brain damage. Odell, (1977)[1] states that subtle cognitive dysfunction may appear in infants with serum unconjugated bilirubin levels of 200 mmol/litre (12 mg per 100 ml). Szymanski *et al* (1978)[3] in a study of 150 children having developmental delay in the neuro-psychological sphere showed that 17.3 percent were group A children of group O mothers compared with 10.1 percent in the general population. This difference was shown to be statistically significant ($p = 0.04$), and demonstrates the need for continuing investigation of ABO HDN.

Four thousand six hundred and seventy-two infants delivered at the Taranaki Base Hospital and the Opunake and Waitara Maternity Annexes during the period 1 January 1974—31 December 1977, are included in this study. This figure does not include infants who were born to mothers who had antibodies in their serum other than the expected antibodies of the ABO system. "Abnormal babies" in this study are those whose cord blood has a positive Direct Coombs Test (DCT) or where the serum bilirubin has exceeded 167 mmol/litre (10 mg per 100 ml) in the neonatal period or both. Infants with hyperbilirubinaemia from other causes such as physiological jaundice, infection and hereditary haemolytic anaemia have not been excluded as in some cases the aetiology is difficult to define.

Methods

ABO Grouping: was carried out by sedimentation technique using cells and serum in tubes. *Direct Coombs Test:* (DCT) — one drop of four times washed cord cells in a 3 percent

suspension plus one drop of anti-human globulin (Ortho). Centrifuge at 3,000 r.p.m. for 15 seconds and read over a concave mirror. *Bilirubin estimation; Bilirubinometer AO 10200* (American Optical Corporation).

Elution

The heat elution method of Lansteiner and Miller was used on all cord cells demonstrating a positive Direct Coombs Test.

Results and Discussion

Table I — *Maternal/Infant Blood Group Combinations*

Table II — *Abnormals.*

Voak *et al* (1969)[5] in a detailed study states that the minimum criteria for the diagnosis of ABO HDN are the serological demonstration of incompatible anti A/B antibodies in an eluate prepared from the baby's red cells, accompanied by the clinical observation of jaundice or more rarely, pallor due to anaemia, in the infant during the first few days following birth.

Positive DCTs in this study were all due to immune anti A or B as shown by elution of the appropriate antibody from the cord cells of A or B infants delivered to group O mothers. The increased percentage of abnormals for group A or B infants of group O mothers in this study can be attributed to the presence of a Positive DCT of the cord cells. If the figures in Table II (61 and 28) are eliminated from the abnormals for this group (Mother Group O in Table I) then the percentage for this group (A or B infants of Group O mothers) is reduced to 15.29 percent, a similar percentage to the other combinations, indicating that it is unlikely that ABO HDN has occurred in the absence of a positive DCT in this study.

Although it has not been possible in this study to exclude infants with hyperbilirubinaemia due to causes other than ABO HDN 14.85 percent of all infants had hyperbilirubinaemia in the absence of positive DCT and could be said to be due to "other causes". It could also be stated that of the 28 babies with both raised bilirubin and a positive Direct Coombs Test 4.158 (14.85 percent) would be expected to have a raised bilirubin regardless of the positive Direct Coombs Test. This means that 24 babies could be said to have clinical ABO HDN able to be detected by laboratory testing, that is, 0.153 percent of all births or 3.19 percent of all A or B infants delivered to group O mothers. A total of 14 babies in this study required one or more exchange transfusions, five of these were A or B babies of group O mothers, however, only two were in the group with both a positive DCT and raised bilirubin, and could be classified as having severe ABO HDN. The 61 newborn with a positive DCT although not clinically jaundiced probably do have a mild haemolytic process. Mollison (1972)[3].

Although this paper does not offer any solution to the problem of prediction of the disease it does demonstrate the probable incidence of the disease in a mixed European/Maori population with the following distribution of ABO groups:— A 42.5 percent, B 8.8 percent, AB 2.7 percent, O 46.0 percent.

Table I

Mother	Baby	Total	'Abnormal'	%
O	O	1,412	204	14.44
	A	617	166	26.90
	B	135	38	28.14
	AB	-	-	-
A	O	586	78	13.31
	A	1,250	206	16.48
	B	53	5	9.43
	AB	56	9	16.07
B	O	151	17	16.83
	A	59	9	15.25
	B	167	28	16.76
	AB	52	7	13.46
AB	O	-	-	-
	A	61	11	18.03
	B	56	5	8.92
	AB	18	0	0.00
Total		4,672	783	16.75

Table II

Raised bilirubin only	694	14.85%
Positive DCT only (no clinical jaundice)	61	3.30%
Raised bilirubin and positive DCT	28	.60%

Acknowledgments

I am indebted to the many laboratory staff who assisted with compiling the figures used in this study.

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Obituaries

James Alfred Samuel

Alf Samuel died in the Mater Hospital, Dunedin, last July. He had submitted to throat surgery earlier in the year. He was born and bred in Dunedin, attending Andersons Bay Primary School and Otago Boys' High School. He was born in 1913. While in hospital I got him to write and record for me on tape some details of his adventuresome life in the Pacific. His schooling took place in the thirties and he recounted the overcrowded conditions in the schools and the preponderance of orphans whose parents succumbed to the 1919 'flu' epidemic. This must have been a feature of this period because I too recall the orphanage children at school at this time. After an office job and a stab at University he appeared to find employment in the University Bacteriology Department at the ripe age of 24. He obtained his C.O.P. and in the ensuing years had the chance to go to some of the Pacific Islands including Pukapuka or Danger Islands somewhere north of the Cook Islands, population 250! This was related to a University Research Programme. Eventually he succeeded to the post of Laboratory Superintendent in Suva taking over from another Dunedin man, Eric Johnston, who was there for some 20 years. By coincidence, Alf was also there for 20 years and came back to Dunedin about 1975. He worked in the Otago Polytech for a time until his illness. He was one on his own. His talents included a sound knowledge of acoustics and sound systems and he was responsible for the design of the original sound system in the Red Lecture Theatre in the Otago Medical School. He was a great raconteur and I can remember his talk at one of the Auckland Conferences about the small-minded squabbling and parochialism of an early New Zealand settlement which turned out to be the early history of that very city! I do not think he would mind if I remarked by way of an epitaph that he was, 'a bit of a dag!'.

R.D.A.

Arthur Francis Bell

The death occurred in Auckland on 27 March of Arthur Bell.

Mr Bell commenced work at the Medical School in Dunedin in 1929 and came to Auckland in 1932 where he joined the staff of Auckland Hospital Laboratory and started his training for C.O.P. in medical laboratory practice which he completed in 1936. During this time he also gained his B.Sc. degree at Auckland University. In 1936 he left the staff of Auckland Hospital and joined Dr Fowler as a technologist in his private practice remaining with Diagnostic Laboratory until his retirement in 1976.

During this time he saw and kept up with many changes both in the development of the Laboratory and in advances in laboratory technology. Prior to his retirement he was involved mainly with cytology.

Mr Bell was a keen sportsman, he played cricket for Auckland and was a golfer and bowler.

J.B.

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Reminiscences

J. A. Samuel

Paraphrased from his written notes and the recollection of many conversations.
R. D. Allan

I started work in the Bacteriology Department of the Otago Medical School in 1937 after spending some years in desultory pursuits. I hoped to have the chance to do some useful work of a practical nature in a scientific environment. I had not spent my time profitably at school due to overcrowded classes, poor teaching and inherent laziness. The incentive of a job with a "training programme" stimulated me to apply myself and at this late stage I obtained Physics I and Chemistry I part-time through the Workers Education Association. The University as represented by the Medical Staff in the Medical School were hardly ideal employers and I was made to work in the animal house for a long period and wherever the vagaries of my employers decided. Training was accomplished by individual effort, dodging down to the other departments for odd periods. My relation with the technical staff were much more satisfactory and such people as Andrew Logan appeared by contrast courteous, helpful and principled. Gilmour, Lynch, Pearson, Hercus and D'ath who I worked for were alsod the examiners for the C.O.P. and it would not have been prudent to object at being pushed from pillar to post and having one's ideas and suggestions used without acknowlegement or due credit. it appeard to me that intellectual dishonesty was almost a way of life in the University. Certainly the people I dealt with were unscrupulous and showed a blatant disregard for the rights or welfare of their staf. As far as salaries were concerned the University authorities behaved like early 19th Century mill owners!

When the Universal Basic Wage came in, a whole year elapsed before the University scrooges could bring themselves to instigate it! In writing these notes at the editor's request, I have experienced a surprising sense of relief at the opportunity to voice these matters. I think I may claim to be of a placid disposition and I was not consciously aware of harbouring resentment all these years.

Andrew Logan was a Scot from Edinburgh. His appointment to the technical post was not uneventful because his initial application did not apparently produce a reply. Some months later he discovered that a reply offering him the job had been sent to his laboratory, opened and concealed by his employer, an eminent bacteriologist who did not wish to lose him. This seemed about par for the course! He left by the next boat! Andrew Logan was a decisive character and a competent bacteriologist. I remember him getting a phone call from a clinician regarding a possible case of gonorrhoea. The slide and the request form were lying near the phone. "Just a moment," he said. In a minute, the slide was stained and examined and Andrew was back on the phone saying, "Yes, there are gonococci there!".

In gram staining there is no need to waste time. I remember demonstrating this fact with the aid of known cultures to Sir Charles Hercus and a class of students. Another useful skill of the period passed on by Andrew Logan was in the early identification of meningococci. In the early nineteen forties a military camp had an outbreak of meningococcal meningitis. A single coccus with a diffuse edge was the typical finding rather than the bean-shaped diplococcus in polymorphs. This had to be distinguished from leucocyte fragments. At one stage I assisted a Viennese doctor who prided himself on his command of English. He wanted a supply of horse blood and I undertook to write to Wallaceville for it. Dissatisfied with my polite circumlocutions, he revised my note so that it became a simple order to send blood by air every Monday. Some days later he was observed pacing the floor in some agitation and on enquiry held out the reply. "Dear Doctor," it said, "If your need is so urgent, why don't you buy a horse!"

Due to a transient virus infection as a child, my hearing of high frequencies was affected and cut off sharply at 12,000 cycles. In spite of this I became very interested in tape recording and managed to secure the apparatus some time before 4YA. I worked with a bacteriologist whose hearing was normal but who had managed to qualify in this branch of medicine although completely red-green colour blind. This became apparent when he examined Ziehl Neelson films or failed to recognise pneumococci on a blood plate.

On first going to high school I joined the camera club and enjoyed all the wonders of quarter plate film packs and enlarging. In a year or so I became allergic to metol developer and had to forsake the darkroom. I did, however, retain my interest in photography and reached the stage

of making what I suppose was a documentary on libraries in Dunedin.

I was able to make use of these skills and in one instance assisted in the planning of the Hercus Building and the Red Lecture Theatre acoustics.

In 1945 I was asked to act as locum laboratory superintendent in Suva to let the incumbent Mr E. P. Johnston have a well-earned six months post-war leave. Ten years later I succeeded him in this post which I found both challenging and satisfying. In the interim I gained the reputation, possibly undeserved, of being an expert on expeditions to the Islands and took part in University projects to Samoa and Pukapuka. My advice was simple. "Take everything you need." Blow lamps were efficient bunsens and a pressure cooker for an autoclave. A set of tools, solder and silver solder and all containers. The only bottles likely to be found on the islands were beer bottles.

When I arrived at Pukapuka I was asked if I had any five inch nails. The island's only boat had been damaged on the reef and the natural knees were broken and had to be replaced. We did not have five inch nails but three inch nails topped and tailed and brazed with silver solder, did the trick! This particular excursion to Pukapuka was made in 1955 during the University vacation. The journey was by air to Apia and then a cruise of an estimated 2½ days duration in the "Ranui" a vessel built in Stewart Island. On this occasion the day broke sullenly and the sky was dark. We sailed off in these threatening conditions and the sky got blacker by the minute. Soon we were being lashed with rain and buffeted by strong winds. We were engulfed in a hurricane coming from the North! The captain vouchsafed no information comforting or otherwise as he battled the storm. He had no radio reports to assist him. We passed the hurricane with winds about 45 knots — quite enough for me!

Four days later we made landfall! It was this storm that damaged the island boat.

When I was appointed laboratory superintendent to the laboratory in Suva Hospital I was told that the pathologist was a typical English gentlewoman. I found Dr Minnie Gosden just that. The best boss I have ever worked with. She had come from a banking family and had done medicine against her father's will. Her first job in the colonial service was in Cyprus and as a young woman she had to perform postmortems out in the open fields under the watchful eyes of armed tribesmen. The colonial service was a wonderful institution; after all the British had been at the game for a long time. You knew your exact field of work, you knew where you stood, and there were rules for everything. The rules had a certain pliability and I was able to acquire three badly needed microscopes. I got permission for one, got a second through the chemical stores fund and a rap over the knuckles and the third out of supplementary estimates! One major hazard to laboratory apparatus was humidity which caused shorting and other troubles with electrical gear. Gentle heat in the form of 15 watt lamps round the spectrophotometer kept it working. Lenses and optical instruments grew moulds. I once took the door off a Bolex 16 mm camera to find the whole of the inside a solid mass of green mould! Electrolytic corrosion on power-points was another problem.

When I was a boy, the Little Sisters of the Poor cart used to call at our bakery for bread, each Friday after shopping in town. The horse a creature of habit used to micturate at this point. In Fiji we got 100,000 unit ampoules of CSL penicillin and one of my jobs was to distribute this into smaller volumes for use. When I opened the ampoule I was powerfully reminded of the little green cart and the horse. It looked and smelt exactly like horse urine!

Incidentally the "Little Sisters" I knew as a youngster were all tiny Frenchwomen who spoke good English in very quiet whispering voices. Imagine my surprise on visiting the Leper Colony on Makogia to hear sisters in the familiar black habit speaking loudly in strong American accents or broad "Strine".

In order to pursue my hobby of tape recording, in addition to the actual recorder I had to pack an engine-driven alternator and hundreds of yards of cable. I recorded church choirs in villages and played them back to their huge delight! I recorded traditional music and the drums of Bara Bara. On one memorable occasion at a function at the Suva School of Medicine I recorded the improvised action song of one Remissio, a student from the Solomons. It was about a driver's conversation with his motor car which kept stopping. The lines included such gems as:

"Why you engine stop do you look at the scene?"
"Engine all right need to look for benzine."
"Why you not go? What is it now?"
"Not go up road fright of bulamakau!"

One of the pleasures of the job was that of selecting staff who were keen to learn. It was exciting to teach them to become competent practitioners. I treated them as equals. We had many difficulties to overcome and improvisation was the name of the game. The Suva steamer was

one example. (Samual 1980)[1]

On one occasion Lautoka Laboratory was given a German blood bank refrigerator by a local firm as a gift during hospital week. I got an urgent call to say it was not working. The instruction book was in German but the local doctor's German wife could not understand it. It turned out that technical German is a closed book to the lay person.

I got the book anyway and laboriously translated it with the aid of a dictionary. It didn't help much so I proceeded to strip down the apparatus. When I got to the air-circulating fan, the trouble was revealed. The fan was the wrong way round on the shaft! This was soon fixed. On another occasion we required a 20°C incubator and as the ambient temperature is always well above this figure in Fiji, we had to find some way of feeding cold water into the apparatus. An old deep freeze was fitted with a ballcock to keep it filled with water and set to run at 10°C. A small stream of this water was siphoned through the incubator with the thermostat set at 20°C. This worked well.

In latter years funds became available for apparatus which was not always well chosen or suitable for the climate. Automated equipment was a case in point. Air conditioning is not an unmixed blessing because if only some of the rooms are air conditioned it is more unpleasant passing from one temperature to the other than remaining in the natural atmosphere.

After retiring, following the granting of independence to Fiji, I returned to New Zealand and looked around for some interesting occupation. I took a job at the Otago Polytech for a while. One of my duties was to look after the class microscropes and I observed that as a regular thing they were so adjusted as to make observations well nigh impossible. It occurred to me that some simple instructions on how to get the best out of the visual microscope would be useful for both students and tutors! Much time and effort seemed to be wasted on a consideration of Kohler Illumination and critical illumination. It seemed to me that the student would be better off if he or she had never heard of either. Subsequently I prepared some notes on a practical nature which were accepted with some alacrity and immediately zeroxed. I hope they proved useful.

Addendum

Alf Samuel prepared these notes and provided me with other details verbally during his terminal illness. Latterly he found writing too tiring but persevered with the aid of a tape recorder to provided some essential biographical details. I believe this project gave him a great deal of satisfaction. He was pleased and proud to be made a life member of the NZIMLT and made arrangements for the document to be framed.

— **R. D. Allan**

Reference

1. The Suva steamer: An example of Alternative Technology. Samuel, J. A. (1980), *N.Z. J. med. Lab. Technol.* **34**, 19.

Book Reviews

Review of Clinical Laboratory Methods. K. L. Mukherjee, M. T. (ASCP), Ph.D., 1979. Published by C. V. Mosby and obtained from N. M. Peryer Ltd, Christchurch. Soft cover, 221 pages, illustrated. Price: $NZ15.90.

The following comments were received from the charge technologists in the various disciplines.

This small book devotes some 63 pages to **urinalysis and clinical chemistry.**

According to the author the purpose of the book is to serve as an overview to the subject. Brief physiological descriptions are given with brief outlines of some methods. The depth of knowledge contained in this book would not be sufficient for trainees in this country. There are a number of annoying simplifications, for example, Henderson Hasselbach equation stated as "pH = pK + log salt/acid." instead of pH = **pKa** + log salt/acid.

The Haematology section of this book provides a simplistic overview of methods and diagnostic procedures which might be useful to a first year trainee or laboratory assistant. I certainly could not recommend its purchase by any trainee in New Zealand. On page 12, fig. 1-7, it is indicated that blood is sucked into a Westergren pipette — no warning is given that this should not be done by mouth!

Immunohaematology or Blood Bank Operation p. 38-60. I feel that this section of the book would have very limited use in a routine blood bank. The

only use it could have would be as a basic introduction to be read by a student during their first week in the Section.

The Author states in the preface that "This book was developed to provide a concise overview" and that its purpose is " . . . avoiding unnecessary details that have little use in actual practice." Very little information is given on the other blood group systems which this reviewer feels have a lot of use in actual practice. No mention is made of hepatitis testing of donors or precautions necessary in a Blood Bank.

The microbiology section of this book is concisely written, easy to read and follow with only a couple of minor mistakes.

Although the book is only intended as a quick review of the subject, the lack of any depth makes it unlikely to be of much use except to the very new student to the subject. The subject matter is only very introductory and the student will soon move on to more advanced introductory texts.

A.G.W., A.E.K., B.W.M., B.M.L.

Review of Medical Microbiology 14th Edition. Jawetz, Melnick and Adelberg. Published by Lange Medical Publications and obtained from N. M. Peryer Limited, Christchurch. Hard cover, 593 pages, illustrated. Price $NZ21.00

Now in its 14th edition, this popular textbook continues to occupy an important position on the laboratory bookshelf.

There are many additions including mention of *Legionnella pneumophila,* Elisa techniques free living amoeba and *Clostridium difficile.* However, *Campylobacter fetus* is not yet included as a human pathogen.

In regard to the virology section the text is not substantially altered from the previous edition. Two additional techniques are outlined namely Immune Adherence Haemagglutination Assay (IAHA) and the enzyme-linked Immunosorbent Assay (ELISA). The principle of these two techniques are briefly described and would not alone constitute a reason for purchasing the 1980 edition. However, this text continues to give a thorough and readable theoretical base and should be strongly recommended to any microbiology candidate interested in proceeding in Virology.

This particular copy reviewed had one notable fault in that the spine had broken with normal usage!

B. M. Lockwood, Elizabeth Poole

Selected Methods in Cellular Immunology (1980) Edited by Barbara B. Mishell and Stanley M. Shiigi (several authors). Published by W. H. Freeman & Company, San Francisco, and obtained from N. M. Peryer Ltd, Christchurch. 486 pages, illustrated, hard-backed. Price $NZ43.50.

The reviewer found this a particularly difficult book to review as it is composed almost entirely of methods and no student of Immunology could pretend to know or understand the intricacies of each and every method.

This is the first edition of a book that started out, in a more humble form, as a teaching manual for undergraduate students at the University of California.

The editors are perhaps a little modest, or at least cautious, in the use of "selected methods" in the title, for this book contains most of the principal methods used by cellular immunologists today.

The list of contributors is impressive and a number will be known to readers. John Marbrook of Auckland rates a special mention for his work (which is included) on the diffusion culture system and the acrylamide raft technique.

The book is divided into four major sections.

I. In Vitro Immune Responses. Included here are the preparation of cell suspensions of all types; their enumeration and tests of viability, the generation of humoral responses, haemolytic plaque assays, cell mediated cytolytic responses, limited dilution analysis and cell proliferation in response to mitogens.

II. Cell Separation using, adherence techniques, size and density measurements, cell surface markers (including rosetting) and mitogens.

III. Preparation of Immunoglobulins for Cellular Studies including the preparation and testing of antisera, purification of immunoglobulins and their fragments, and modification and use of antibodies to label cell surface antigens.

IV. Additional methods. This section covers such topics as RIA (Radioimmune Assay), selected surgical procedures (experimental animals), hybridoma technique, and polyacrylamide gel electrophoresis.

The book has as well a comprehensive list of abbreviations and a detailed appendix covering the preparation and testing of a useful array of every-

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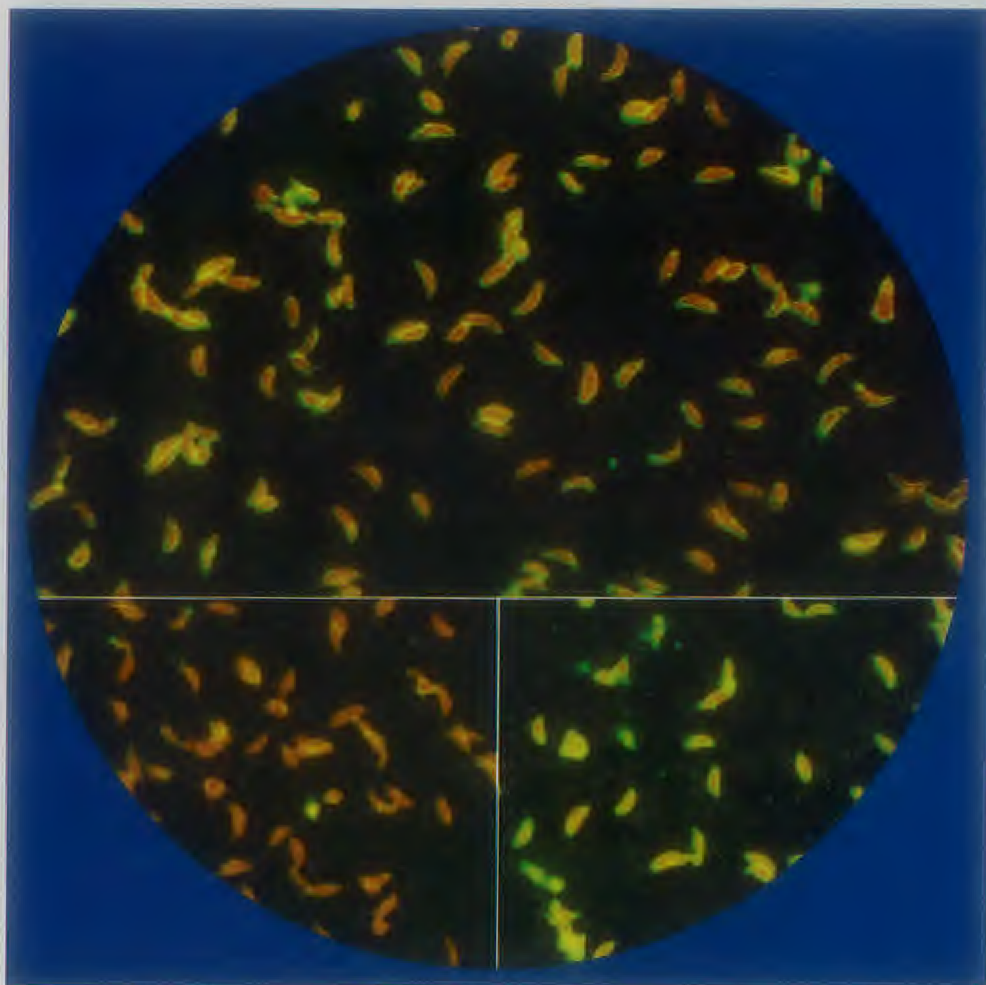

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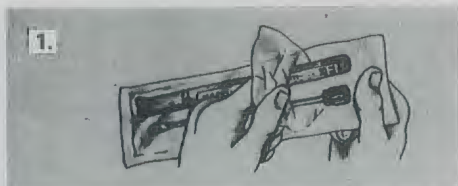

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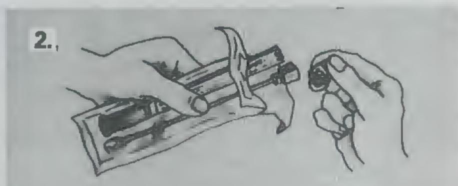

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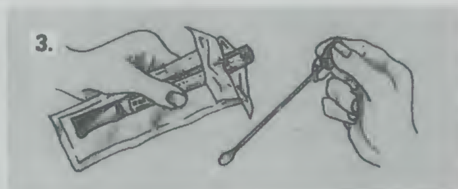

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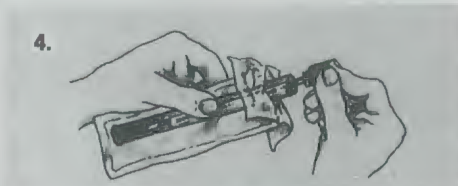

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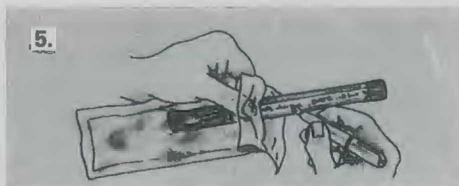

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day reagents, the proper preparation of glassware for tissue culture work, and so on. Each method is described in detail mostly under the following categories, Materials and Reagents (including supplier, *in the USA*), procedure, comments, and references.

As well, the major and minor topics all have comprehensive introductions, and the comments section (when included) for the most part is detailed and most helpful to the understanding of the technique in hand.

This type of book is appealing in that methods are contributed by individuals or groups of individuals who use these methods on a day to day basis and for this reason one expects and gets exact details of procedure.

Selected Methods in Cellular Immunology is not intended for bedtime reading, but is a serious attempt to bring together otherwise widely scattered methodologies. It is intended mainly for students and research workers in the various fields of Immunology where they have ready access to experimental animals, however a large private or hospital clinical Immunology laboratory might find a number of these methods suitable if not adaptable to their needs.

G. S. Elliot

Abstracts

Contributors: Margaret Berry, E. R. Crutch, Shirley Gainsford, N. J. Langford and L. M. Milligan.

CLINICAL BIOCHEMISTRY

An Improved Continuous Flow Method for Serum Creatinine Using the Jaffe Reaction. White, W. A. and Attwood, E. C. (1980), *Ann. clin. Biochem.* **17**, 153.

A method is described in which serum is dialysed directly into a combined alkaline picrate reagent, resulting in increased precision and elimination of baseline noise and drift.

— N.L.

Improved Electrophoretic Separation of Creatine Kinase Isoenzymes. Burlina, A. (1980), *Clin. Chem.* **26**, 317.

C.K. isoenzymes are separated on cellulose acetate with use of an improved procedure for Reactivation and Visualisation. The reactivator is N-Acetyl Cysteine and 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyl 2-H-tetrazolium bromide for visualization in a tetrazolium-coupled system. Said to be both reliable and practical, and a suitable alternative to chromatographic or immunochemical assay.

— N.L.

An Assessment of the Corning Electrophoretic Procedure for High Density Lipoprotein Cholesterol Estimation. Bullock, D. G., Carler, T. J. N., Cosgrove, C. M. and Hughes, S. V. (1980), *Ann. clin. Biochem.* **17**, 148.

The results correlated well with those obtained by phosphotungstate/Mg precipitation and ultracentrifuge procedures, and showed acceptable accuracy and linearity, but precision is less satisfactory at low levels of HDL cholesterol. The procedure requires great attention to detail, with care needed in timing and set up of the densitometer.

— N.L.

Rapid Determination of Alpha-fetoprotein in Amniotic Fluid Using an Automated Immunoturbidmetric Technique. Evans, S. E. (1980), *Ann. clin. Biochem,* **17**, 130.

Described is a rapid and precise immunoturbidmetric assay on a Centrifichem 400 Centrifugal analyser, for the measurement of alpha-fetoprotein in amniotic liquor. The assay is able to distinguish 4 mg/l from zero and is unaffected by gross blood staining in the liquor.

— N.L.

Immunoglobulins in Cerebrospinal Fluid in Various Neurologic Disorders Bouloukos, A., Lekakis, J., Micheal, J. and Kalofaitis, A. (1980), *Clin. Chem.* **26**, 115.

It is proposed that determination of levels of IgG . A . and M in CSF is of greater significance than the relative immunoglobulin content.

— N.L.

Decreasing Sample Volume Used in the "Sequential Multichannel Analyser Computerized" (SMAC). Stamper, R. and Robertshaw, D. M. (1980), *Clin. Chem.* **26**, 778.

Modifications to the alkaline phosphatase and creatinine cartridges on the 18 channel S.M.A.C. decreases the sample size and eliminates the problem of fibrin clots in the stream-splitter on the main sample line. Accuracy, precision, linearity and carryover are acceptable after these modifications when compared with premodification data.

— N.L.

Interference of Imferon in Colorimetric Assays for Iron. Huisman, W. (1980), *Clin. Chem.* **26**, 635.

Imferon, an iron-dextran complex is used in patients with iron deficiency. In contrast to saccharated iron, it disappears slowly from the circulation. In the presence of the various reducing agents used in colorimetric assays for iron the dextran-iron complex decomposes and the freed iron becomes dialysable, and can react in the iron assay.

— N.L.

HAEMATOLOGY

An International Collaborative Study Establishing a Reference Preparation for Antithrombin III. Kirkwood, T. B. L., Barrowcliffe, T. W. and Thomas, D. P. (1980), *Thrombos. Haemostas. (Stuttgart)* **43**, 10.

An international collaborative study involving 12 laboratories in seven countries was carried out to establish a suitable reference preparation of antithrombin III. This material has been established by the World Health Organisation as the International Reference Preparation for antithrombin III, with an assigned potency of 0.9 i.u. per ml.

— E.R.C.

Disseminated Intravascular Coagulation. Findings in 346 Patients. Spero, J. A., Lewis, Jessica H. and Hasiba, U. (1980), *Thrombos. Haemostas. (Stuttgart)* **43**, 28.

An analysis was made of 346 cases of disseminated intravascular coagulation utilising a combination of laboratory tests. The authors felt that the following six tests best reflected the pathophysiology of DIC:— Platelet count, fibrin split products, fibrin monomers anti-Thrombin III levels, peripheral blood smear and fibrinogen.

— E.R.C.

Lineation of the Osmotic Fragility Curves of Erythrocytes. Try, K. (1980), *Scand. J. Haematol.* **24**, 157.

This author suggests that the osmotic fragility curve may be described by two values — the concentration of which 50 percent of the erythrocytes are haemolysed and the decrease in concentration raising the fraction of haemolysis from 0.50 to 0.80. This study indicates that by lineation, the procedure of the osmotic fragility testing can be simplified to using only 2 buffered saline solutions, with no great loss of precision or accuracy.

— E.R.C.

A New Form of Ehlers-Danlos Syndrome. Arneson, Mary A., Hammerschmidt, D. E., Furcht, L. T. and King, R. A. (1980), *JAMA* **244**, 144.

The authors describe the clinical and laboratory findings in this disorder and comment on the correction of the defect with fibronectin.

— E.R.C.

Comparison of Three Methods for the Estimation of Plasma Antithrombin. McLellan, D. S., Devlin, J. D., Heyse-Moore, G. H. and Aronstam, A. (1980), *J. clin. Pathol.* **33**, 438.

Plasma antithrombin levels were measured by clotting, immunological and amidolytic methods on normal individuals and post-operative patients. The authors discuss the merits of each method.

— E.R.C.

Hand - Mirror Lymphocytes in Infectious Mononucleosis. Thomas, W. J., Yasaka, K., Strong, D. M., Woodruff, C. M., Stass, S. A. and Schumacher, H. R. (1980), *Blood,* **55**, 925.

The findings of increased numbers of hand-mirror lymphocytes in patients with infectious mononucleosis and their correlation with the recovery phase of the disease suggest that these cells have a role in the self-limitation of infectious mononucleosis. The morphology of these cells is distinctly different from the classical description of atypical lymphocytes of Downey.

— E.R.C.

Identifying Carriers of Mild Haemophilia. Graham, J. B., Barrow, Emily S., Flyer, P., Dawson, Deborah V. and Elston, R. C. (1980), *Br. J. Haemat.* **44**, 671.

The problems of carrier identification in mild haemophilia were examined by studying a large kindred. The authors found that the factor VIII coagulant assays were almost as informative as the paired factor VIII antigen/factor VIII coagulant assays.

— E.R.C.

Clinical Laboratory Determination of Antithrombin III. A New Semi-automated Method. Brandt, J. T. and Senhauser, D. A. (1980), *Am. J. clin. Pathol.* **73**, 687.

The authors present a new semi-automated method for the determination of antithrombin III. They utilise the chromogenic substrate S-2238 and the Abbot ABA - 100.

— E.R.C.

Preparation of lyophilized Abnormal Haemoglobin Controls for Cellulose Acetate Electrophoresis. Proksch, G. J. and Bonderman, D. P. (1980), *Am. J. clin. Pathol.* **74**, 64.

A process for the preparation of stable lyophilized normal and abnormal haemoglobin controls for alkaline cellulose acetate electrophoresis is described. The materials appear to have an extended shelf life of at least two years at 5°C.

— E.R.C.

Normal and Abnormal Bleeding Times in Neonates and Young Children Utilising a Fully Standardised Template Technique. Feusner, J. A. (1980), *Am. J. clin. Pathol.* **74**, 73.

The standard adult template method was modified for use with infants. The bleeding time with this technique for healthy term (3.4 min ± 0.9) and preterm (3.6 min ± 1.0) newborn infants did not differ from that for healthy young children (3.4 min ± 1.3).

— E.R.C.

Acute Lymphoblastic Leukaemia. Hand Mirror Variant. Schumacher, H. R., et al. (1980), *Arch. Pathol. Lab. Med.* **104**, 134.

The presence of numerous hand mirror cells in the bone marrow of patients with acute leukaemia may be related to a good prognosis. To further evaluate this relationship, the bone marrow of 21 consecutive patients with a diagnosis of acute lymphoblastic leukaemia were reviewed for the presence of greater than 40 percent hand mirror cells. Although not statistically significant, the authors' findings suggest a positive relationship between the presence of greater than 40 percent hand mirror cells in the bone marrow and a favourable prognosis.

— E.R.C.

An evaluation of the Hemalog 8: Three Years' Experience. Bourne, G. G. and Scott, T. J. (1980), *Aust. J. med. Lab, Sci.* **1**, 1.

A description of the principles of operation of the Hemalog 8 is presented along with experience and problems encountered over a three year period. A number of modifications were necessary to ensure reliable operation. The machine is now extremely useful in automatically providing a complete Haematology profile including platelets.

— E.R.C.

IMMUNOHAEMATOLOGY

Brush Up Your Medicine: Blood Transfusion. Isbister, J. P. (1980), *Med. Jnr. Aus. May, 3, 1980.*

The author discusses changing attitudes towards Blood Transfusion therapy. Clearly outlined are some of the basic risks involved in routine and emergency cross-matching.

— L.M.M.

Platelet Concentrates for Transfusion: Control of Production and Storage. Tandy, N. P., and Taylor, M. A. (1980), *Med. Lab. Sciences,* **37**, 2.

To derive maximum benefit from transfusion of platelet concentrates optimum conditions of preparation and storage must be maintained. A number of in vitro techniques have been tested to achieve this. Optimum yields were obtained by carefully monitoring centrifugation and storage conditions.

— L.M.M.

Advantages of Low Ionic Strenth Saline (LISS) Techniques in Blood Bank Management. Haigh, T. J., and Fairham, S. A. (1980), *Med. Lab. Sciences,* **37**, 2.

The use of low ionic strength saline solution (LISS) as a suspending medium for red cells, enables blood to be cross-matched on demand in

less than 30 minutes. The ensuing reduction in unnecessary cross-matches has led to a 25 percent reduction in the blood bank workload and a 19 percent reduction in blood wasted by outdating.

— L.M.M.

Low-Ionic Strength Media for Rapid Antibody Detection: Optimum Conditions and Quality Control. Noah, D., Downie, D. M., Damborough, J., Haigh, T., and Fairham, S. A. (1980), *Med. Lab. Sciences,* **37**, 2.

Observations are presented on the use and quality control of low ionic strength saline (LISS) in agglutination and spin anti-globulin tests. A number of false positive reactions are discussed.

— L.M.M.

Hemophilia: Cost Consideration for Prescribing Therapeutic Meterials. Linney, D. R., and Lazerson, J. (1979), *Trans.* **19**, 1.

Costs for therapeutic products utilised in the treatment of hemophiliacs can be prohibitive. The costs can effectively be reduced by understanding the costs involved. By calculating appropriate dosage schedules, the total financial burden can be reduced and valuable therapeutic material conserved.

— L.M.M.

Expression of HLA-DR Antigens on T Lymphocytes. Gerlase-Deliria, M., Kraemer, M. H., Nao, N. F., Paua, E. R., and Mendes, N. F. (1980), *Tissue Antigens,* **15**, 257.

HLA-DR antigens were detected by microlymphocytotoxicity employing defined and non-defined anti-HLA-DR sera. The HLA-DR T cell antigens proved to be the same as the ones expressed on B cells from the same donor. Extra reactions were observed on nitrogen-stimulated and on T cells which could be abolished by absorption with cytotoxicity negative B cells of the same donor.

— L.M.M.

Methyldopa Inhibition of Suppressor-Lymphocytic Function. Kirtland, H. H., Mahler, N., and Horwitz, D. A. (1980), *New Eng. Jnr. Med.* **302**, 15.

To test the hypothesis that methyldopa induces red-cell auto-antibodies by inhibiting the activity of suppressor lymphocytes, its effect on several immune functions was studied. Methyldopa inhibited T-lymphocyte suppression of IgG production by peripheral blood mononuclear cells which had been stimulated. The drug caused 30 percent to 80 percent reduction in the proliferative response of peripheral-blood mononuclear cells to mitogens in vitro — this reduction primarily involved the activation of T lymphocytes. These and other associated events, may lead to unregulated autoantibody production by B cells in some patients.

— L.M.M.

Increased Incidence of Haemolytic Disease of the New-Born caused by ABO-Incompatibility when Tetanus Toxoid is given during Pregnancy. Gupte, S. C. and Bhutia, H. M. (1980), *Vox Sang,* **38**, 22.

There was a significant increase in the incidence of neonatal jaundice among ABO-incompatible infants of women given toxoid, compared to a control group. Further comparative studies, suggest an increased risk of haemolytic disease of the new-born due to ABO incompatibility when tetanus toxoid injections are given during pregnancy.

— L.M.M.

An Example of "Naturally Occurring" Anti Js^a(K6) in a Japanese Female. Ito, K., Mukumoto, Y., and Komishi, H. (1979), *Vox Sang,* **37**, 350.

Anti Js^a was found in a Japanese female who had never received a transfusion and borne 3 Js^a-negative children. The antibody agglutinated Js^a cells suspended in saline and treated with proteolytic enzymes as well as reacting with these cells by the indirect antiglobulin technique. This example is the first reported naturally occurring Anti Js^a.

—L.M.M.

Anticomplement and the Indirect Antiglobulin Test. Wright, M. S., and Issitt, P. D. (1978), *Trans.* **19**, 6.

A large number of IgG complement-fixing blood group antibodies of 17 different specificities were used in order to determine whether anticomplement antibodies are still necessary in antiglobulin reagents to be used in antiglobulin tests. It was concluded that anticomplement antibodies are essential for the correct performance of indirect antiglobulin tests.

— L.M.M.

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MICROBIOLOGY

Rapid Biochemical Characterisation of Haemophilus Species by Using the Micro-ID. Edberg, S. C., Melton, E. and Singer, J. M. (1980), *J. clin. Microbiol.* **11,** 22.

The Micro ID system was used to distinguish H. influenzae from H. parainfluenzae using the indole and ONPG tests. Using indole production, ornithine decarboxylase, urease production and nitrate reductase the same system was used to biotype H. influenzae into 5 types and H. parainfluenzae into 3 types. 90 percent of cerebrospinal fluid isolates of H. influenzae were biotype I and 70 percent conjunctival isolates were biotype II.

— S. G.

Perinatal and Neonatal Infection, (1979), *J. antimicrob. Chemo.* 5, Suppl. A.

This supplement consists of papers given at a symposium and includes Group B streptococcal infections, listeriosis, toxoplasmosis, gram negative rod infections plus others and the routes of transmission of these micro organisms.

— S.G.

Clinical and Laboratory Characteristics of Achromobacter xylosoxidans. Igra-Siegman, Y., Chanel, H. and Cobbs, C. (1980), *J. clin. Microbiol.* **11,** 141.

The authors describe six cases of infection due to A. xylosoxidans including the identification and antibiotic susceptibility of the organism.

— S.G.

Evaluation of the Phadebact Gonococcus Test, a Coagglutination Procedure for Confirmation of Neisseria gonorrhoeae. Lewis, J. S. and Martin, J. E. Jr. (1980), *J. clin. Microbiol.* **11,** 153.

The Phadebact Gonococcus Test was compared with the fluorescent antibody test and carbohydrate utilisation on stock cultures of various Neisseria species and on clinical isolates. It proved to be highly specific and identified 97.1 per cent of isolates.

— S.G.

Assessment of the Till-U-Test GC Slide. Donald, W. H. (1980), *Br. J. vener. Dis.* **56,** 81.

The Till-U-Test GC slide was compared with modified Thayer Martin (TM) medium for the isolation of Neisseria gonorrhoeae in clinical specimens. It was more sensitive than the TM medium and as it provides its own CO atmosphere is a suitable medium for the isolation of N. gonorrhoeae when laboratory facilities are not immediately available.

— S.G.

Comparison of Drug Sensitivity Testing with Microdilution Quantitative Minimum Inhibitory Concentration and the Autobac I System. Jacob, C. V. and Kleineman, J. (1980), *J. clin. Microbiol.* **11,** 465.

The Autobac I system was shown to provide reliable semiquantitative estimates of bacterial susceptibility when compared with a microdilution quantitative MIC system on four hundred common organisms.

— S.G.

Detection Of Specific Anti-Leptospiral Immunoglobulins M and G in Human Serum by Solid Phase Enzyme-Linked Immunosorbent Assay. Adler, B., Murphy, A. M., Locarnini, S. A. and Faine, S. (1980), *J. clin. Microbiol.* **11,** 452.

The ELISA anti IgM and anti IgG techniques were found to be sensitive and specific for detecting leptospiral antibodies in human sera and had some advantages over the microscopic agglutination test.

— S.G.

Rapid Gas Chromatographic Method for Identification of Metabolic Products of Anaerobic Bacteria. Rizzo, A. F. (1980), *J. clin. Microbiol.* **11,** 418.

A simple method for the separation of volatile fatty acids, alcohols, ketones and non volatile fatty acids, and their identification using a single gas chromatographic column is described.

— S.G.

Enzyme-Linked Immunosorbent Assay for Mumps and Parainfluenza Type 1 Immunoglobulin G and Immunoglobulin M Antibodies. Ukkonen, P., Vaisanen, O. and Penttinen, K. (1980), *J. clin. Microbiol.* **11,** 319.

An ELISA for mumps and parainfluenza type 1 antibodies (IgG and IgM) is compared with the complement fixation test. The mumps IgG ELISA was not much more sensitive than mumps CF but the mumps IgM ELISA gave a diagnostic result with one serum instead of having to show a four fold rise in antibody titre with the CF.

Parainfluenza type 1 IgG ELISA was more sensitive than CF but parainfluenza type 1 IgM antibodies were not detected by ELISA when the CF titres were raised.

— S.G.

Significance of Aspergillus Species Isolated from Respiratory Secretions in the Diagnosis of Invasive Pulmonary Aspergillosis. Nalesnik, M. A., Myerowitz, R. L., Jenkins, Rosemary, Lenkey, J. and Herbert, D. (1980), *J. clin. Microbiol.* **11**, 370.

On examination of 78 patient records and x-rays, all of whom had Aspergillus species isolated from their respiratory secretions, the authors found that even a single isolation of Aspergillus fumigatus and Aspergillus flavus from compromised patients was significant. Aspergillus niger was not usually associated with disease.

— S.G.

Bacterial Antigen Detection in Body Fluids: Methods for Rapid Antigen Concentration and Reduction of Nonspecific Reactions. Doskeland, S. O. and Berdal, B. P. (1980), *J. clin. Microbiol.* **11**, 380.

Ethanol precipitation at a subzero temperature with the addition of albumin was used to concentrate bacterial antigens 20X in CSF and urines. After deproteinisation by boiling, serum was concentrated in the same way.

— S.G.

Use of Modified New York City Medium for Growth of Mycoplasma pneumoniae. Granato, P. A., Poe, L., and Weiner, L. B. (1980), *Am. J. Clin. Path.* **73**, 702.

Modified New York City medium (MNYC) was evaluated for its ability to support the growth of *M. pneumoniae*, as compared with the conventional culture medium for Mycoplasma. This medium was capable of supporting growth 5-6 days sooner than by conventional methods. Minor modifications in the basic formulation of the New York City medium (NYC) have been made to produce a suitable medium for supporting the growth of *M. pneumoniae.* The formulation of MNYC medium is documented in this paper.

— M.E.B.

Liquid Medium for Growth of Legionella pneumophila. Ristroph, J. D., Hedlund, K. W., and Allen, R. G. (1980), *J. clin. Mic.* **11**, 19.

A simple yeast extract broth capable of supporting the growth of *Legionella pneumophila* is described. Rapid growth of *Legionella pneumophila* is produced in this liquid medium from a small inoculum. Using carcoal yeast extract agar requires a large inoculum and on primary isolation growth is slow. Yeast extract broth also provides a system for investigation of antigens and possible toxins. The formulation and preparation of yeast extract broth are described.

— M.E.B.

Grouping of Beta-Haemolytic Streptococci by Agglutination. Easmon, C. S. F., Cox, S. E. M., and Howard, A. (1980), *J. clin. Path.* **33**, 386.

This paper examined the practicability, in a busy routine laboratory, of adapting commercial Streptococcal grouping sera for use in agglutination tests. Particular attention was paid to ease of adaptation, reliability, ease of reading and cost. Streptococcal grouping sera, after having been diluted and absorbed to remove cross reactions, were bound to Staphylococci and used to group trypsinised beta-haemolytic Streptococci by coagglutination. Results were compared with those obtained using the Phadebact Streptococcal grouping kit. It was shown that it is technically possible to adapt Streptococcal grouping sera for use in coagglutination tests by a series of dilutions and absorptions, and although time consuming, large volumes of coagglutination reagent may be prepared from a small quantity of the original grouping sera at a relatively low cost.

— M.E.B.

Diagnostic Clinical Parasitology (1) Proper Specimen Collection and Processing. Garcia, L. S., and Voge M. (1980), *Am. J. Med. Tech.* **46**, 386.

This article is the first of a series of four papers on diagnostic clinical parasitology. It presents various collection methods, the preservation of specimens, and deals with diagnostic procedures including direct smears, concentration techniques and permanent stained smears. The information presented includes the pros and cons of each method discussed. Topics included in the next three presentations will include protozoa, helminths and blood parasites.

— M.E.B.

Serologic Immunodiagnosis of Invasive Aspergillosis. Holmberg, K., Berdischewsky, M., and Young, L. S. (1980), *J. Inf. Dis.* **141, 656.**

Two sensitive serological techniques, Counter-immunoelectrophoresis (CIE) and Enzyme Linked Immunosorbent Assay (ELISA) were used for detecting antibodies to Aspergillus, in a study from patients with histologically proven invasive aspergillosis. Serial specimens showed conversion from negative to positive immunoprecipitin reactions and changes in ELISA titres during immunosuppression. Positive CIE reactions were demonstrated in 70 percent of patients with clinically suspected aspergillosis while ELISA detected 80 percent. In addition to being more sensitive, serial antibody determination by ELISA allowed for separation of seropositive patients into two groups. A serial rise in ELISA titre appeared to correlate with histologically documented recovery from infection, whereas those with declining or persistently intermediate titres were found to have disseminated aspergillosis at autopsy. The reagents and methods are discussed.

— M.E.B.

Evaluation of Cerebrospinal Fluid Lactic Acid Levels as an Aid in Differential Diagnosis of Bacterial and Viral Meningitis in Adults. Lannigan, R., MacDonald, Margeret, A., Marrie, T. J. and Haldane, V. E. (1980), *J. clin. Microbiol.* **11**, 324.

Contrary to some reports these authors did not find that the concentration of lactic acid in cerebrospinal fluid (CSF) was reliable enough to differentiate bacterial infection from viral. False positive and false negative results occurred and it was decided that the measurement of lactic acid in CSF had no advantage over standard tests.

— S.G.

Rapid Presumptive Identification of Vibrios by Immobilisation in Distilled Water. Chester, B. and Poulos, E. G. (1980), *J. clin. Microbiol.* **11**, 537.

Vibrio and Campylobacter species were tested for motility in trypticase soy broth (TSB) and distilled water by suspending a colony from an 18 hour sheep blood agar plate in the broths then examining them microscopically for motile organisms. The Vibrio and Campylobacter species were motile in TSB but not in distilled water. A variety of other gram negative bacilli were motile in both.

— S.G.

Identification of Toxigenic Clostridium Difficile by Counterimmunoelectrophoresis. Welch, D. F., Menge, Sue K. and Matsen, J. M. (1980), *J. clin. Microbiol.* **11**, 470.

A counterimmunoelectrophoresis (CIE) technique was compared with a cytotoxicity test in the detection of C. difficile toxin from culture filtrates. The sensitivity of both methods was similar but positive CIE results were also obtained with C. bifermentans and C. sordelli isolates.

— S.G.

Evaluation of Rapid Carbohydrate Degradation Tests for Identification of Pathogenic Neisseria. Pizzuto, D. and Washington II, J. A. (1980), *J. clin. Microbiol.* **11**, 394.

156 isolates of Neisseria were tested by the modified rapid fermentation test (MRFT), the BACTEC Neisseria differentiation kit and the cysteine trypticase agar (CTA) method. The CTA method was the most accurate method but it required 48 hours of incubation. The other methods were found to be nearly as accurate and required only 2-3 hours of incubation.

— S.G.

ERRATUM

N.Z.J. med. Lab. Technol. (1980), 34, 29 paragraph 4. 1-α-thalassaemia should read, 2-α-thalassaemia. In the last sentence of the same paragraph, α chains should read γ chains.

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