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T. H. Pullar Memorial Address 1979

Back from Basics to the Tea-Lady

Dr Michael B. Gill,
Head, Auckland Hospital Board School of Medical Laboratory Technology.

An Address to the NZIMLT Conference, Auckland 1979

I will begin by thanking you very sincerely for the honour you have done me in inviting me to deliver the 13th Thomas Pullar Memorial Address.

I never had the privilege of knowing Dr Pullar—I began training in 1967, the year after his death, but by reading the previous memorial addresses one can build up a picture of him as someone aware of the scope and fascination of laboratory medicine, and someone who showed warmth and concern in his relations with people, whether they were patients or his colleagues in the laboratory. It is clear, too, that he had the vision to see a new profession, that of medical laboratory technology, in the making, and he did his utmost to help that profession become established and grow in strength.

I have looked back over the addresses given by the previous 12 speakers and noted with interest the changes that have occurred. To begin with all the speakers were pathologists but of late there has been a healthy predominance of technologists. The assumption, either expressed or implied, that only pathologists should manage laboratories has been mentioned less often of late and then with just a hint of anxiety. The traces of condescension, faint but discernible, present in some of the earlier addresses have gone and it was a pleasure to read in 1978 the assertiveness of the young Kennedy. Even last year the use of the adjective young in this context was questioned but I will keep to it—we young people must stick together.

A month ago when I was casting about for a theme for this address, two important events were taking place. The first was the mid-year meeting of the Medical Technologists Board, the first I had attended, and the second was the Auckland Film Festival. I tried to combine these two events into an imaginative tale symbolic of our times. There was the Australian film *The Chant of Jimmy Blacksmith* and I could see this transmuted into a New Zealand setting under the title *The Chant of Harry Hutchings*— the story of a highly-motivated young man who, goaded beyond endurance, runs amok with a mortuary knife, killing six pathologists.

Then there was the award-winning film, *Padre Padrone* and I could see this as *Padre Desmondo Phillipone*— the story of a rural laboratory, idyllic in its setting but surrounded by huge buildings whose inhabitants have broken legs and cleft palates. The story shows the despairing attempts of the venerable bearded principal of the laboratory to acquire an auto-analyser to replace his time-honoured methods based on witchcraft and the movements of the sun. It ends tragically when he has still had no reply from the Health Department after 15 years waiting.

From these dizzy heights I descended, with a terrible clunk, to the title *Objectives in Laboratory Medicine.* Management, which used to be called leadership in earlier times, is very much in vogue these days, though the achievement of good management has so far proved elusive in the New Zealand health system. Nevertheless, its increasing importance is not questioned and the first step in good management is to define one's objectives.

This is not easy.

We could aim to build the biggest laboratories in the Southern hemisphere, an objective that looked reasonable in 1959, or even 1969, but in 1979 it is undesirable as well as being impossible for financial reasons. A more worthwhile objective, one which was dealt with in Mr Kennedy's address last year, would be to build up the most effective laboratory service possible within our current economic constraints.

In the end I have chosen as a simple and facile objective, that we should aim to enjoy ourselves. Like all simple facile answers it begs the real question which is how we should enjoy ourselves.

Money is part of it but I will steer carefully around this subject except to make the single comment that I believe the senior management positions in technology should have their pay scales lifted higher than they are at present. If I were given the authority, I would make the position of Director of a laboratory equally accessible to

technologists, science graduates or pathologists. The appointment would be a three year one, renewable if the person in the position remained the best available but not automatically renewable, and this would allow the unsuitable Director to be fired out after only three years of damage and someone else given a chance. It has always seemed to me that a medical degree and a post-graduate qualification in pathology do not automatically endow abilities of leadership; nor does a university degree; nor does a Certificate of Proficiency. Nor does any of these qualifications automatically prevent a person from being a good manager. It depends on the individual. Such a three-year term of office, lacking any security of tenure, would need to get a high salary. It would be an exciting departure from our present system but I would have to admit that flexibility and acceptance of insecurity are not really part of our national character.

For the rest it seems that technologists' pay scales are relatively fixed and if you really want to become a millionaire then you must toss in the security of a hospital laboratory and take up some high risk entrepreneurial activity such as collecting and marketing agar from the East Coast or marketing kiwi fruit in Rhodesia or live sheep in Iran.

What else do we need to enjoy ourselves? We need to feel a sense of achievement and feel it at reasonably frequent intervals. A pre-requisite of achievement is that one must have been given some sort of responsibility, which is usually described in management terms as delegation of authority. This need not be authority of a major variety—it might be just the responsibility of identifying the aetiology of an anaemia for an anxious house surgeon when you are by yourself on night call, or making the glucose method work correctly for the first time, or taking the responsibility for training a first year laboratory assistant. But it must be some sort of responsibility and it must be placed on people right from the beginning.

On the subject of delegation of authority let me read to you a paragraph from a book which is the best of any I have read on management. It is called "Up the Organisation" and the author is Robert Townsend. This is what he writes on delegation of authority:

> "Many give lip service, but few delegate authority *in important matters.* That means all they delegate is dog-work. A real leader does as much dog-work for his people as he can: he can do it, or see a way to do without it, 10 times as fast. And he delegates as many important matters as he can because that creates a climate in which people grow."
>
> The page before this is on Decisions. He says: "All decisions should be made as low as possible in the organisations. The Charge of the Light Brigade was ordered by an officer who wasn't there looking at the territory."
>
> He goes on: "There are two kinds of decisions: Those that are expensive to change and those that are not."
>
> "A decision to build the Edsel or Mustang shouldn't be made hastily . . .
>
> "But the common garden variety decision—like when to have the cafeteria open for lunch or what brand of pencil to buy—should be made *fast.* No point in taking three weeks to make a decision that can be made in three seconds—and corrected inexpensively later if wrong. The whole organisation may be out of business while you oscillate between baby-blue or buffalo-brown coffee cups."

Let me contrast that style of writing with that in the May 1979 issue—the first issue—of Management Training News which distributes information on management to New Zealand Hospital Boards. Here is how they start to summarise the principles of a nationally co-ordinated plan for the year 1979.

"1. Everyone on the staff who manages or supervises others—or who may do so—needs and is entitled to planned training opportunities to help him or her to manage more effectively.

2. The line-manager is the basic trainer in any organisation. Special training staff may support or supplement their efforts through planned programmes; but this plan aspires to acknowledge, support and strengthen the exercise of that fundamental line-management responsibility to develop staff competence.

3. Normally, management training will be planned as a multi-disciplinary programme, focussing on the common skills required for managing by the various disciplines and professions involved, and emphasising the collaborative management style that is needed throughout the entire health care service."

I am not quite sure what all that means. I am sure it is well-intentioned. I am sure they will have no trouble getting through their $195,000 budget for the 1979-80 year. I will be very surprised indeed if it results in more effective management in the laboratories where we work. For what is wrong is not the training of people, but the system, which decrees that decisions shall be made as high as possible in the organisation, not as low as possible.

In our laboratories, even the most minor decisions on purchasing and staff must often be made by the Hospital Board. The larger decisions go to the Health Department and a surprising number seem to go to the Minister himself. We all know the enormous amount of wheel-spinning that goes on as we write our long and persuasive letters as to why such-and-such a piece of equipment should be purchased knowing full well that we may not actually need the equipment at the moment but since it will take at least three consecutive annual submissions to get it, we have to start now. We all fill vacant positions as soon as possible in case they should be closed down by the Sinking Lid mechanism. We know all sorts of areas of waste which we would not allow if we had personal control of the budget.

I believe there is only one management decision the Health Department should make, and that is the total laboratory budget for each Hospital Board.

And for their part, the Hospital Board should make only one management decision, and that is how that budget will be split between the individual laboratories under their control. After that, the way the budget is spent should be entirely in the hands of the laboratory.

I hasten to add that these decisions about how to split the budget are the big ones for they concern the vital question of priorities in health. But that is what senior management is for, to make the big decisions, not to decide whether Auckland Hospital should be allowed a data-capture device for its 12/60, or whether Thames should have two graded positions or three, or whether technologists should be allowed to accumulate duty leave. These decisions should be placed squarely on the laboratory people themselves.

With the current spate of interest in management, there is a danger that we will simply enlarge our bureaucracies and make them even less productive. As a terrible example we need only look at the re-organisation of the health services in Britain in 1974, widely regarded as a failure, because of the top-heavy administrative superstructure which includes three tiers of decision-making. As a result, decisions go into a loop, perpetually circulating between the three tiers. Decisions are finally made by a process of attrition or chance, and that is not the way decisions should be made.

If ever we do adopt the policy of delegation downwards, senior management will become redundant and in the perfect organisation, all decisions will ultimately be made by the tea-lady.

While I am on this subject of management decisions being handed down from a distant bureaucracy, let me mention in passing the Nationwide Clinical Laboratory Computer System which, according to rumour, is in its death throes. A transplant of new hardware and a massive infusion of dollars would no doubt bring it to life again but there is something wrong with our management when this is all we have to show after spending one-third of 23.7 million dollars. Mr Townsend writes one of his better paragraphs on institutional management consultants: "They are," he says, "disastrous. They waste time, cost money, demoralise and distract your best people and don't solve problems. They are people who borrow your watch to tell you what time it is, and then walk off with it."

There is a move afoot to make management teaching part of the Part II syllabus for COP. To me it is doubtful whether someone as busy as a Part II student, should be loaded down with a subject as nebulous as management is, or can be, for this is the sort of subject that should be studied at post-graduate level. The proposed management syllabus includes 1100 pages of reading and I suggest you think carefully about that before accepting it. When I browsed through the syllabus I was interested to note that the book on first aid recommends the use of smelling salts, which are not widely used in modern medicine.

I will use my last quote from Robert Townsend as a bridge to the last section of this address when I will talk about education, a subject which is almost compulsory for someone who has the title, Head of the Auckland Hospital Board School of Medical Laboratory Technology. The quotation under 'T' for Training reads "The only way I know to get somebody trained, is on the job." My experience of education consists of an extended medical training and the three year period when I was Chief Examiner in Chemical Pathology for COP. As I look back at the medical course I had inflicted on me, I am amazed at the zeal of those who fed totally useless information into me and at the sheer volume of it all. There is an article on this subject in the Lancet entitled "A Mythology of Medical Education." The author attacks a number of myths, and in particular the myth of the Basic Sciences—which reads, he says, "like some gloomy Norse Saga . . . . This myth states that no student can deal with a problem until he has studied "the basic sciences"—until he has "an adequate foundation." But in the event, he says, the foundations are all too often not only inappropriate for the super-structure they are meant to bear, but so monolithic and huge in design that they will never be finished in time. When the student enters clinical practice the foundations are

necessarily incomplete and so poorly related to his needs that they remain a rapidly crumbling ruinous mass on which he must erect a temporary structure to cope with the forceful reality of practice.

Historically one can trace this myth to the mid-19th Century when little was known about any aspect of medicine except anatomy. It was possible then to emcompass the whole scientific knowledge known to man and still have time in the curriculum left over—time was filled with the study of Latin. You can count yourselves lucky that the NZCS course was laid out in the 1960's rather than the 1930's, else you would have found yourselves saddled with a unit of Latin. It was assumed that the Latin somehow exercised and developed the mental musculature, like some intellectual body-building course, so that one could thereafter study anything with greater ease and effect. That particular assumption has now been shifted to chemistry and biochemistry but the reasoning behind it is similar and its protagonists are equally unshakable in their beliefs.

Let me make it clear that I am not against including any basic science in the medical technology syllabus—my argument is only with the particular subjects selected and the belief that the coverage of the basic sciences can in any way be comprehensive. In the 1930's there began the accelerating explosion of knowledge which is so large now that no-one can hope to realise even its scope, let alone understand it all. In the 1930's Krebbs and his co-workers discovered those elegant biochemical cycles that we have all worked through, like the labours of Hercules. I remember almost nothing of my five terms of biochemistry except one piece of information from our very precise little professor of biochemistry, "This enzyme", he said, "is found in only two situations, in the milk of lactating mammals and in the pineapple. But no-one has yet seen a pineapple suckling its young."

What basic sciences should we have in the NZCS syllabus? Since the war there have been enormous advances in pharmacology, immunology, endocrinology, cellular and molecular biology, behavioural and social psychology, and most of our knowledge of clinical biochemistry and haematology has been built up since that time. Computer technology is progressing at an astonishing rate so that in our own work increasingly, we simply add a sophisticated pre-mixed reagent to our sample and take the result from a computer printout. All of these sciences are relevant and basic—there is so much that might be useful, and so little time for them.

Most would agree that the basic methodologies of the main departments must be taught, in depth, but after that, should the bias be towards the pure sciences such as chemistry biochemistry and molecular biology, or should it be towards the applied medical sciences such as the study of disease processes, which is called general pathology, and the interpretation of laboratory tests which is clinical pathology? My own inclincation is towards the pathology. Pure science and electronic technology are being taken over by the instrument manufacturers who supply their products in almost push-button form. Interpretation is still in the hands of a relatively small and overworked medical profession many of whom welcome expert assistance. The pathologist is the traditional interpreter of laboratory tests but there are not a lot of pathologists around and many of them are histo-pathologists. If you can establish with the physicians and surgeons and GP's of your area that you know more about the interpretation of a test or group of tests than the pathologist does, then I say good luck to you.

Why should we worry about the content of NZCS? It worries me because the time of our students is valuable and the attitudes they develop are important. From what I have seen, our students are not stimulated by much of the present syllabus.

Parts of the first two years are boring or irrelevant or both—and they need not be. We have good students, highly selected, and laboratory medicine is a fascinating subject. I suggest that we should try to improve the syllabus.

To me this is the greatest merit of the proposed Diploma course, that it will provide a better syllabus. I doubt whether the structure of the course with its blocks of full-time study is necessarily better than the present one. For all its disadvantages, I think the apprenticeship-type of training is better for most people than a full time course. I remember one of the Technical Institute tutors saying how much more enthusiastic and better motivated our medical laboratory technology students are than those who are doing full time science courses at the same institute. The pattern of mixing real work in the laboratory with formal tuition in the technical institute seems to me a sound one. It should be the pattern of the whole of our professional lives.

And that raises the problem of post-graduate education, a problem which will not be solved by the proposed Diploma. When technologists have completed their COP's or Diplomas, what goals can they set themselves? The only goal that can

easily be recognised at present is the slow climb up the grading ladder.

My own belief is that a qualification requiring a thesis is what we should be aiming for. There is of course the Fellowship of the Institute but, it seems, your members rarely bother with it. I have heard of only a few theses being presented in Auckland since the Fellowship began some 12 years ago and a recent one was shot down by an eager assessor who obviously wanted to "raise the Standards" as the saying goes. This reminded me of a 19th Century New Zealand bird collector called Reishek who made a collection of skins of the native stitch-bird. He decided to increase the value of these skins by exterminating the remaining stitch-birds, which he systematically did, shooting all except a remote few still left in the ravines of Little Barrier Island. In the same way it is common practice to maintain the rarity of qualifications by trying to exclude others from obtaining it. I personally believe that admission to Fellowship by thesis should be encouraged and given greater recognition rather than making it appear unattainable as it might seem now.

The purpose of education, I believe is to train people to be both critical and constructive and a thesis can achieve this in a way that no exercise in rote-learning can. The candidate is given a problem (which can be something of a common, practical nature) and they must look for the answer. He or she must look closely at current methods, read the literature, delve into the relevant basic sciences, make observations, and draw conclusions—at the end of it all they should be able to think, write and speak clearly about their work. And that is what education is about, not regurgitating pages of formulae and definitions that have been painfully committed to memory and almost instantly forgotten.

The problem with the Fellowship is that it is not recognised widely in the way that a university Masters degree is or a PhD. What one would like the university to do is accept the NZCS or COP as equivalent to a BSc and this would mean that the technologist with ability could proceed with a Masters degree and later a PhD. At present the University gives little credit from an NZCS towards a BSc, but I suspect some improvement could be negotiated if your Institute felt that this was worthwhile.

Of one thing I have no doubt, and that is that your bright students need some sort of additional goal to aim for beyond the COP—or Diploma—and for my part I would give it higher priority than the proposed new Diploma.

On that note I will finish. In brief I would like to see an undergraduate NZCS or Diploma course more interesting, more stimulating and more relevant than the present one. I would like to see a post-graduate qualification that will provide a challenge for the most able students. And finally a delegation of real responsibility down from the Hospital Boards and Health Department into the laboratories where the workers live. Given all that, I think our laboratories could be more exciting places.

I would thank you again for inviting me to deliver this address and wish you an exciting and successful Conference.

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Obituary

James Marr 1933-1979

Jim grew up in Whangarei and was a school teacher before commencing training in 1960. He qualified in 1966 and subsequently worked at Princess Mary Hospital before returning here as Deputy Principal Technologist and charge Technologist in Haematology—Blood Bank. He made a major contribution to the development and co-ordination of the Blood Banking Service through Northland.

He took two months leave without pay, to upgrade the Blood Transfusion Service in Western Samoa and, in 1974, served with the New Zealand Surgical team in Vietnam.

In 1976, he left to take charge of the Blood Bank at Prince Henry Hospital, Sydney.

He was interested in hockey, captained the Whangarei colts, played as a Whangarei senior representative and managed Northland Mens' teams for several years until 1976.

The full church at his funeral was evidence of the high regard in which he was held in the community.

—K.B.R.

A Comparison of Red Cell Antibody Elution Methods

Jan Hamer and Keiry Kennett

Immunohaematology Department, Christchurch Hospital

Received for publication, February 1979

Summary

A survey was conducted to compare the elution of 11 different examples of human IgG antibodies against red cell antigens, D, c, E, K, Fy^a, Fy^b, Jk^{ab}. The four different methods used were: heat, ether, digitonin and acid. All eluates obtained were titred and scored.

Introduction

Elution methods are necessary to obtain antibodies from cells sensitised either *in vivo,* for example in Haemolytic Disease of the Newborn or auto-immune disorders, or *in vitro* where antibody mixtures are found and it is necessary to perform adsorption and elution studies on a serum to characterise individual antibodies.

A method for routine use in the general blood banking laboratory should be quick, easy to perform and use reagents which are easily obtainable.

In past years various methods have been developed and modified (4, 7, 8, 9, 10, 14, 16, 18, 19, 20). Of these acid, heat and ether methods have been compared by Rekvig and Hannestaad (1977)[14], heat and formalin freeze methods by Greenwalt (1956)[4], and ether and formalin by Rubin (1963)[16]. A review of different methods giving full technical details has been published recently by Ford (1978)[3]. The commonly used methods heat, acid, ether and digitonin have not been compared directly for their yield of antibody. To establish which method was preferable each technique was used to elute 11 different examples of IgG antibodies.

Materials and Methods

Human IgG antibodies obtained from Christchurch voluntary donors were used. Sensitisation was effected by mixing equal volumes of packed red cells and antisera and incubating at 37°C for 60 min. Cells were then washed in ice cold normal saline six times to comply with conditions of the Rekvig and Hannestaad method, although normally washing is carried out at room temperature. Following sensitisation a direct Coombs test was performed to ensure antibody uptake by the red cells.

Elution Methods

(1) Acid Elution—Rekvig and Hannestaad (1977)[14].
Add equal volumes of 50 percent cell suspension and ice-cold pH 3.0 glycine HCl buffer, incubate in an ice-cold water bath for 1 min. Centrifuge for 30s at high speed, remove the supernatant immediately and adjust the pH to 7.0 with 20µl/ml of eluate. Use tris 0.5M buffer pH 10.0.

(2) Heat Elution—Landsteiner and Miller (1925)[10].

(3) Ether Elution—Rubin (1963)[16].

(4) Digitonin Elution—using Gamma ELU-KIT, Lot 114.

For details of these last three methods readers are referred to the review by Ford (1978)[3].

The eluates obtained were titred using the antiglobulin method and all sensitisations, elutions and titres were performed in duplicate.

Results and Discussions

Comparing the scores of the eluates obtained, the ether method gave the highest yield with the majority of antibodies (Table 1). Digitonin was the next most useful for good antibody yields followed by acid and heat.

However, other factors must be considered for suitability as a routine laboratory method. The ether method gives a deeply haemoglobin-stained eluate which poses no problems when using the antiglobulin technique for further investigations, but causes difficulty when techniques used do not involve a washing process. In addition to this disadvantage, the reagent used, di-ethyl ether, is volatile and dangerous to store in the laboratory.

The digitonin method is available in kitset form (ELU-KIT) from Gamma Biologicals (kindly supplied free of charge by Medic DDS.) Sufficient quantities of each reagent are supplied for about 22 elutions. Instructions for use are provided with the kit which are clear and easy to follow. A problem with this method is in the initial wash to remove the haemoglobin-stained supernatant, where it is difficult to see the stroma layer. Consequently some of the stroma may be lost accidently in the washing process thus reducing the resultant antibody yield. This can be overcome by using a swing-out head centrifuge for packing the stroma, rather than a fixed-head centrifuge which packs the stroma against the side of the tube. The final eluate is haemoglobin free and therefore of use for a wide range of methods. Digitonin is a known

heart accelerant and should be treated as a dangerous chemical.

The acid elution method is quick and easy to perform but requires an ice-cold waterbath and preparation of own reagents. The eluate is haemoglobin free. The heat method is simple and quick and again eluate is haemoglobin free, but antibody yield is poor (Table 1).

During this study it was noted that even though sera of high anti-Duffy titre were used to achieve sensitisation and a direct Coombs test of at least 2+ obtained, the eluates showed a low antibody yield.

One Duffy antibody did not elute. With four of the antiy-Fy^a sera used, ether elution gave the best yield and in one case digitonin gave the best yield. However, these eluates were considerably less potent than other IgG antibodies tested.

There is little previous documentation for this phenomenon of difficulty in elution of Duffy antibodies. Baker *et al.* (1956)[1] were unable to elute anti-Fy^a from the cells of a neonate suffering from Haemolytic Disease of the Newborn and Greenwalt (1956)× showed a reduced yield of anti-Fy^a from sensitised red cells.

To facilitate better yield of Duffy antibody from sensitised cells, modifications were made to two of the methods.

(1) The acid elution method gives a supernatant eluate which is twice as dilute as the other methods. The possibility that the Duffy antibody was present but so dilute as to be undetectable was considered. For this reason packed cells were used instead of a 50 percent cell suspension as suggested in the original method, before the addition of an equal volume of glycine-HCl buffer. This modification showed a two fold increase in titre with an anti-Kell eluate. However, using two anti-Fy^a sera no detectable antibody activity was found in the eluates.

(2) Directions supplied with digitonin ELU-KIT state that washing continue until stroma are nearly white and wash saline no longer haemoglobin stained, as inadequate washing may cause lysis of test red cells. To obtain white stroma as many as six washes were necessary and it is possible that anti-Fy^a may have been dissociated and lost during the numerous washes, which were also time consuming. The digitonin method was repeated therefore using anti-D and anti-Fy^a washing the stroma only three times but did not show improved antibody yield. The resultant eluate did not lyse the test red cells.

As two modifications above afforded no better elution of the anti-Fy^a it seemed that an investigation of the fate of the anti-Fy^a in the elution procedures may aid in discovering a more suitable method. Several theories are possible for the fate of the anti-Fy^a:

(1) It remains firmly attached to the red cells and thus needs a stronger elution method.

(2) Antigen-antibody complexes are removed together and free antibody is therefore undetectable in the eluate.

(3) The antibody is successfully eluted but denatured by the chemicals used.

The latter two theories have been cited previously by Fong and Masouredis (1967)[2] and proof of either of the two happening involves complicated biochemical and immunological techniques, Fong and Masouredis (1967)[2], Kochwa and Rosenfield (1964)[9].

Further investigations of the first theory, that harsher and more thorough methods are needed, were undertaken. The methods used were:

1. Digitonin-Ether Schanfield[18].
   To 10 ml of 10-20 percent washed red cells add 10 drops of digitonin (5mg/ml in saline). Mix for 10 min. Centrifuge 10-15 min and resuspend in saline. Repeat wash process until the stroma is white and the supernatant is clear. Add equal volumes of 22 percent albumin to packed stroma followed by 1 volume of ether equal to the volume of albumin, mix 1 min. Incubate at 30 °C for 30 min, centrifuge 10 min. Remove ether layer with pipette and drive off excess ether at 37 °C for 15 min.
2. Ether-Acid (our own modification).
   The ether-acid method involves the preparation of a normal ether eluate. After two washes in normal saline the packed stroma is then resuspended in a volume of pH 3.0 glycine-HCl buffer equal to the starting volume of red cell suspension and left for 5 min. 1 drop of pH 10.0 Tris buffer per ml of eluate is added for neutralisation. The suspension is centrifuged and the supernatant eluate removed.

Results of the two methods show an increased yield of the same anti-Fy^a sera used in the earlier methods (Table II).

The latter two methods afforded a four-fold increase in yield of anti-Fy^a compared with the ether method or the digitonin method. These results also show a difference between anti-D and anti-Fy^a. Anti-D is completely eluted by the ether method as no further antibody could be demonstrated in the eluate obtained by further elution of the stroma with acid. Whereas by this

Table 1.—A Comparison of Elution Yields

Antibody Specificity	Original Serum Titre	Eluate: Heat Titre	Heat Score	Acid Titre	Acid Score	Digitonin Titre	Digitonin Score	Ether Titre	Ether Score
*D	64	4	16	8	24	4	8	32	46
D	64	0	0	8	26	4	14	16	31
c	8	1	3	0	0	0	0	4	13
E	16	1	5	2	6	2	8	2	10
E	16	2	8	8	16	4	16	16	21
K	100	0	0	16	41	16	41	4	21
K	100	0	0	16	34	32	45	16	35
*Fy^a	64	0	0	0	0	0	0	2	8
Fy^a	64	0	0	0	0	0	0	2	8
Fy^a	32	0	0	0	0	0	0	2	8
Fy^a	32	0	0	0	0	0	0	2	8
Fy^a	16	0	0	0	0	2	8	0	0
Fy^a	16	0	0	0	0	2	8	1	3
*Fy^a	4	0	0	0	0	0	0	0	0
Fy^a		0	0	0	0	0	0	0	0
Fy^b	32	1	3	0	0	0	0	0	0
Fb^b	32	0	0	0	0	0	0	1	3
Jk^a	4	0	0	0	0	0	0	4	13
Jk^3	2	0	0	0	0	0	0	1	3

Duplicate results are shown in all cases except for Jk^a, Jk^3 and c where there were insufficient sera.
*Sera used for results shown in Table II.

method more anti-Fy^a is eluted off the stroma by acid following ether treatment, than by the use of ether alone.

These results prompt the suggestion that it may be necessary to employ strong two-stage elution methods to produce antibody activity in the eluates from proven Coombs positive cells when other methods fail or when Duffy antibodies are the suspected cause.

Table II—Elution Yields of Two-Stage Methods

Antibody Specificity	Original Serum Titre	Eluate: Ether Titre	Ether Score	Ether-Acid Titre	Ether-Acid Score	Digitonin-Ether Titre	Digitonin-Ether Score
D	64	32	46	0	0	32	44
Fy^a	64	2	8	8	18	8	18
Fy^a	4	0	0	0	0	0	0

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ABO Antibodies in Cryoprecipitate

Judith M. Wall, Jill A. McKnight and L. M. Milligan

Immunohaematology, Diagnostic Laboratory Services, Dunedin Hospital

Received for publication, March 1979

Introduction

In an earlier report by Aubert *et al* (1941)[1] which was later confirmed by Mollison and Young (1942)[5], it was indicated that A and B group specific substance, present in small

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amounts of plasma and serum, could provoke an immune response in persons whose serum contained naturally occurring Anti A and Anti B antibodies. A number of cases have also been reported by Rosati *et al* (1970)[7], concerning Haemolytic anaemia apparently due to Anti A in Factor VIII concentrates. Work was carried out in Nijmegen[7] concerning the stimulation of antibodies after the infusion with cryoprecipitate.

The following simple study was carried out at the request of the medical staff following a discussion concerning the practicality of introducing a policy of group specific cryoprecipitate infusion. This study, by no means supplies all the answers, but was carried out in order to clarify the following specific questions: 1. does the detectable antibody level remain stable, decrease or increase during processing; 2. what happens to the strength of the detectable antibody where a number of random units are pooled.

Method

The method for processing the cryoprecipitates was based on the finding that Anti-haemophilic factor (Factor VIII) precipitates in the cold with other cryoglobulins of fresh human plasma. Using a slightly modified technique, Pool and Shannon, (1968)[6], 60 units were prepared. Samples were taken from the separated plasma, Factor VIII concentrate, Factor VIII depleted plasma and randomly pooled units. All samples wre subjected to the following tests: 1. Lysin screen test. Carried out using the method described by Mollison[4]. We acknowledge the fact that the lysin test is not accurate, but it was used only as a screening technique. All degrees of lysis were recorded. 2. Titration of Anti A and Anti B: titre was determined according to the method described by Issitt (1975)[2]. A rise or fall in titre of more than two tubes was considered to be significant. Scores were also noted. When units were pooled, equal volumes of each unit were used. Other observations were noted such as weights and volumes of products, but were considered not to be important for the purpose of this exercise.

The pools (Table C) were made up of a random mixture of ABO groups. The six pooled units from the previous day were added to the current day's pool of six (Table D). Thereafter, the same procedure was adopted until a final pool of 60 was reached.

RESULTS

ABO GROUPS

		A	*B*	*O*	*Total*
A Total number of units tested		*22*	*10*	*28*	*60*
Baseline: Mean titre in original specimen	*Anti A*	—	*128*	*128*	
	Anti B	*32*	—	*128*	
Mean titre in Cryoprecipitate	*Anti A*	—	*256*	*256*	
	Anti B	*64*	—	*128*	
Mean titre in Factor 8 depleted plasma	*Anti A*	—	*128*	*256*	
	Anti B	*32*	—	*128*	
B Range of titres obtained in baseline spec.	*Anti A*	—	*4-256*	*16-512*	
	Anti B	*16-256*	—	*8-256*	
		Original Plasma	*Cryoprec-ipitate*	*Factor VIII Dep. Plasma*	
C Random pools of six units each:					
Mean titre of	*Anti A*	*64*	*32*	*32*	
	Anti B	*32*	*32*	*32*	
D Random Pool of:					
6 units Titre of	*Anti A*	*64*	*32*	*32*	
	Anti B	*32*	*32*	*32*	
12 units Titre of	*Anti A*	*64*	*64*	*16*	
	Anti B	*32*	*64*	*32*	
18 units Titre of	*Anti A*	*32*	*16*	*32*	
	Anti B	*32*	*16*	*32*	
24 units Titre of	*Anti A*	*32*	*64*	*32*	
	Anti B	*32*	*64*	*16*	
60 units Titre of	*Anti A*	*8*	*16*	*8*	
	Anti B	*8*	*16*	*16*	

Discussion

(1) The titres of Anti A and Anti B in all three products showed little variation. The Anti A and Anti B from group O donations, showed a slight rise in titre on processing. (2) On pooling, the decrease in the titre of Anti A and Anti B in the pools and especially in the final pool, was largely due to the neutralization affect of the presence of A and B sustance. Only one specimen contained an antibody which caused *in vitro* lysis of the appropriate cells. This lysin was detected in the three products, using recalcified serum, but on pooling was not detected.

The results observed and assessed from the above exercise, did not lead to any significant changes in policy. It would therefore appear, that the decision to infuse group specific cryoprecipitate needs to be based on the requirements of each individual case. Careful consideration needs to be taken concerning the practical availability of group specific cryoprecipitate, the frequency of infusion, the amount to be infused and the quality of the lysin and antibody screening procedure carried out on the original donation.

Wherever practical, cryoprecipitate should be administered on a group specific basis, but if this is not possible, cryoprecipitate pooled from adequately screened donors, could be used for all groups of recipients.

We wish to acknowledge the willing co-operation of the Otago Regional Blood Transfusion Service in providing the donations when required.

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A Review of Low Ionic Strength Solution (LISS) as a Red Cell Suspending Medium

R. J. Austin,

Immunohaamatology Department, Taranaki Base Hospital.

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Summary

In 1967 the Blood Bank at the University Hospital, in Lund, Sweden changed from using Saline as a red cell suspending medium for their crossmatches, to a low ionic strength solution (LISS). Seven years later a paper was published by Low *et al.* (1974)[7] from that Institution describing their experiences with this solution. More than 100,000 units of blood crossmatched with their method had been transfused in this time without any transfusion reactions due to unidentified blood group antibodies. Since publication of their paper many other papers 2, 5, 6, 8, 9, 10 have been published describing the advantages of using this low ionic strength solution or a modification of it. The object of this paper is to review these papers and to introduce additional findings of our own following four years experience in New Zealand with LISS.

Introduction

Red cells suspended in physiologically normal saline are negatively charged due to ionised COOH groups of sialic acid on the red cell membrane. This charge, and the repulsive forces (zeta potential) thus created, results in a gap between individual cells. The ionised groups of both the antigen and the antibody are partially neutralised by oppositely charged ions in physiologically normal saline. If the ionic strength of the medium is lowered, while maintaining its tonicity, the ionised groups would have an increased attraction Ross *et al.* (1978)[9], Atchley, *at el.* (1964)[1], Elliott *et al* (1964)[3] and Hughes-Jones, *et al.* (1964)[4], have all stressed the importance of reduced ionic strength in the suspending medium. Hughes-Jones *et al.* (1964)[4], found that a reduction of ionic strength from 0.17mol to 0.03mol resulted in 1,000 fold increase in the amount of anti D that

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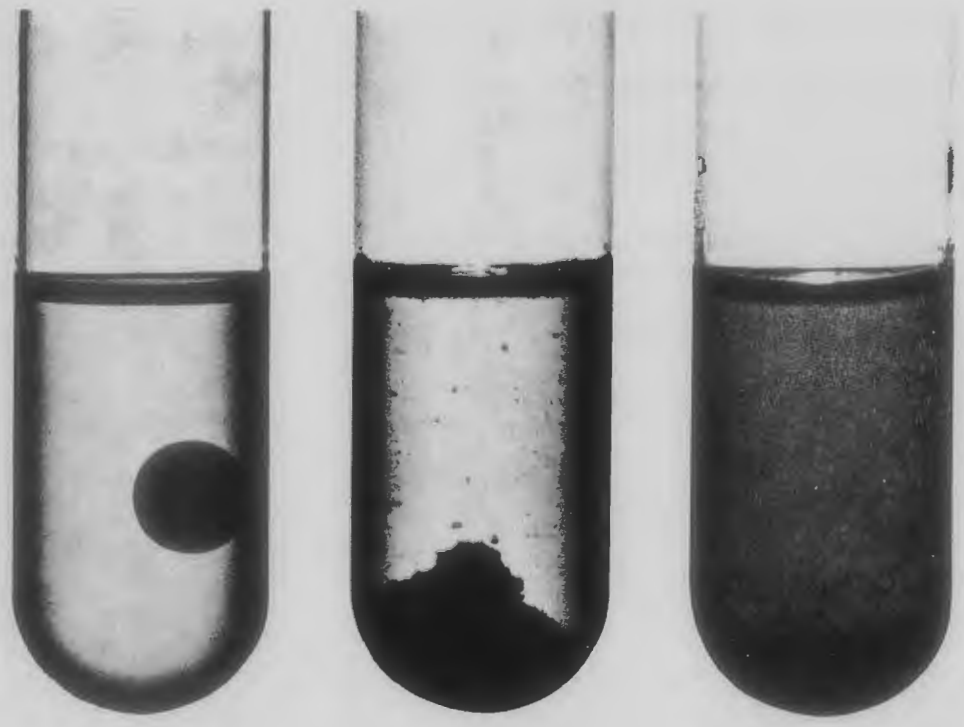

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would bind to D positive red cells in the initial association between antigen and antibody, thus demonstrating an increase in the equilibrium constant (K) resulting in the increase in the sensitivity of agglutination tests and the reduction in the time required for the uptake of antibody by a red cell. Similar observations were reported by Elliot *et al.* (1964)[3] and Atchley *et al.* (1964)[1].

From their observation that the effects of LISS and proteolytic enzymes in enhancing antibody uptake in agglutination, are similar, but not additive, Atchley concluded that both methods worked by effecting a net reduction of the electrostatic charge at, and surrounding the red cell membrane. It is known that proteolytic enzymes actually remove portions of antigen bearing chains from the red cell membrane and in doing so also remove negatively charged residues. The LISS method appears to work by supplying fewer charged ions in the medium than are present in higher ionic strength solutions such as 0.85 percent saline, Issitt *et al.* (1978)[5].

Low *et al.* (1974)[7] found that when red blood cells were suspended in a solution of Sodium Glycinate containing 0.03mol NaCl, buffered to pH 6.7 with phosphate, the incubation time of cells with antibody containing serum could be reduced to five minutes in performing the indirect antiglobulin test and false positive results were of negligible importance. With cells suspended in LISS, compared with cells suspended in saline, positive reactions developed more rapidly and the antibody titre was about four fold greater. Moore *et al.* (1976)[8] showed when using some antisera that even after 1m incubation positive results were obtained with cells in LISS whereas negative results were obtained with cells in saline. In tests using a reference preparation of anti D, the lowest concentration of anti D which could be detected in the indirect antiglobulin test after 15m incubation using saline suspended cells was 0.025mg/ml and using cells in LISS it was 0.005mg/ml. It is apparent that some antibodies are identified with greater ease with the LISS method, others equally well as with the saline method, a definite decrease in sensitivity could not be shown for any antibodies Low *et al.* (1974)[7].

Methods

Low Ionic Strength Solution (0.03mol) LISS

Saline	180ml
Phosphate Buffer 0.15M pH 6.7	20ml
Glycine 0.3M pH 6.7	800ml

Dissolve 18g of Glycine in approximately 500ml of distilled water. Add: 1.0M NaOH drop by drop, (stirring until pH 6.7), 20ml phosphate buffer, 1.79g NaCl dissolved in approximately 100ml distilled water. Make up to 1l with distilled water, dispense into 500ml MRC bottles and autoclave 10lb/20m. Label and store in cold room at 4°C.

Phosphate Buffer

0.15M pH 6.7

Solution A: $NaH_2PO_42H_2O$ 25.05g/1.

Solution B: $Na_2HPO_412H_2O$ 45.25g/1. to obtain pH 6.7 take 9ml of A, 11ml of B and mix.

Quality Control

As no method has been described for controlling the quality of the LISS reagent produced, the following methodology has been evolved at Taranaki Base Hospital and is used for determining the suitability of LISS reagents produced by ourselves and by Biolab (Biological Laboratories Ltd, Auckland).

1. Panel with fresh AB serum at 4°C, room temperature, 37°C by LISS Coombs technique to detect false positive rections.
2. Titre against an antisera of known titre.
3. Test with a serum containing an antibody which has shown to work by LISS previously, but not by saline techniques. (This is achieved by retaining an antisera diluted to this extent).
4. Check for haemolysis by standing at 37°C for one hour with normal washed cells and with enzyme treated cells.
5. Measure pH (6.7).
6. Check osmolality (using an "Advanced Osmometer") Wicker *et al.* (1976)[10]. (An osmolality of 290-320mmol/kg is satisfactory in practice).

Technique

1. Wash cells three times in saline.
2. Re-suspend to 3 percent in LISS instead of saline after final wash.
3. Add one drop of LISS suspended cells to one drop of serum.
4. Incubate mixture at 37°C 10m.
5. Wash four times in saline and add one drop of anti-human globulin.
6. Spin and read macroscopically.

N.B.

Apart from steps two and four, the method is the same as for a normal indirect coombs test.

LISS Crossmatch

All cells used (donor and patients) are washed 3 x in normal 0.17M saline, then re-suspended in 0.03 M LISS to 3 percent.

1. *10m LISS at room temperature.*
   Use 75 x 12mm disposable tubes. Add 1 drop

of patient's serum, 1 drop of 3 percent washed donor cells in LISS and mix well. Leave at room temperature for 10 minutes, centrifuge for 10 seconds and read microscopically.

2. *10m LISS Indirect Coombs.*
   Use 75 x 12mm disposable tubes. Add 1 drop of patients serum, 1 drop of 3 percent washed donor cells in LISS, mix well and incubate at 37 °C for 10m. Centrifuge for 10s and examine macroscopically over a concave mirror for agglutination and haemolysis, if negative wash 4 x in saline decanting last supernatant, add 1 drop of Coombs reagent.
   Mix well, centrifuge for 10s and read over a concave mirror, if negative add 1 drop 3 percent Coombs control cells, spin and re-read.
3. *15 Minute Lows papain*
   Using 75 x 12mm disposable tubes add 1 drop of patient's serum, layer 1 drop of Low's papain. Layer 1 drop of 3 percent donor cells in LISS. Incubate at 37 °C for 15 minutes. *Do Not Mix!*
   Centrifuge for 10s and read macroscopically over a concave mirror. Look for agglutination and haemolysis.

Emergency Crossmatch

Techniques are the same as for a routine crossmatch except that the LISS Indirect Coombs test may be shortened to five minutes incubation if necessary and the room temperature reactions may similarly be shortened if necessary. The Low's papain reduces to 5 minutes incubation.

Notes:

1. As in standard practice, known positive controls should be put up for the Indirect Coombs test and the Low's papain technique using "weak anti D" and prepared R_1r control cells in LISS.
2. It is important to check that each tube is thoroughly mixed prior to incubation in the room temperature and Indirect Coombs tests but not for the Low's papain Method.
3. Ficin works equally as well as papain in this technique.

Discussion

In New Plymouth the LISS method as outlined above, has been in use since 1975 initially on a trial basis and since December 1976 as the routine method in our laboratory for crossmatching, antibody screening, antibody identification and antigen typing. In these four years experience with LISS there have not been any problems caused by the use of the low ionic strength solution.

The false positive problem which was first outlined by Hughes-Jones *et al.* (1964)[4] has not eventuated as it has been overcome by the use of the solution described by Low where the ionic strength of the serum-cell suspension is equivalent to about 0.09mol Moore *et al.* (1976[8]) well above the level where non-specific uptake of protein occurs. Low *et al.* (1974)[7], Moore *et al.* (1976)[8] and Austin (1976)[2] have all emphasized the need for equal volumes of serum and LISS suspended cells to be used. When using other testing systems the antibody-antigen reaction is usually enhanced by having a greater quantity of serum than cell suspension. However, with LISS it is important that equal volumes of serum and LISS suspended cells be used, otherwise the low ionic strength effect is negated. When using glass tubes it is satisfactory to deliver the serum and cells as drops from an ordinary pasteur pipette, however, with plastic tubes which attract drops by an electrostatic force the volume of drops vary too widely Moore *et al.* (1976)[8]. It is recommended by Moore that with such tubes some method such as the use of an Eppendorf pipette which ensures delivery of a fixed volume should be employed. In practice we have found that by ensuring that the tip of the pipette is at least 25mm above the tube the effect of the electrostatic force is nullified and even drops can be delivered.

The effect of adding albumin to the mixture of antibody containing serum and cells has been compared with that of omitting the albumin but suspending the cells in LISS. Moore, *et al.* (1976)[8]. In all cases the degree of augmentation of the reaction by suspending the cells in LISS was far greater than that achieved by adding albumin to cells in saline. In case the combination of albumin and LISS should prove advantageous 30 percent albumin was dialysed against LISS and then used for the re-suspension of red blood cells. One volume of the 2 percent suspension of cells and the dialysed albumin was incubated with an equal volume of serum and the cells tested in the usual way. This method proved to be very insensitive. Moore *et al.* (1976)[8].

When cells were washed in LISS rather than saline before being tested with an anti-globulin serum only a very slight enhancement of the reactions was observed Moore *et al.* (1976)[8]. Austin (1976)[2] and Lincoln *et al.* (1978)[6], have shown that elutions in saline and AB serum tested by LISS gave higher titres and greater avidity than by Indirect Coombs Techniques. Lincoln *et al.* (1978)[6] have also introduced LISS into the absorption stage of the elution procedure as it increases the uptake of the antibody by the red cells. Even though no actual increase in antibody titre may occur almost invariably an increase in antibody

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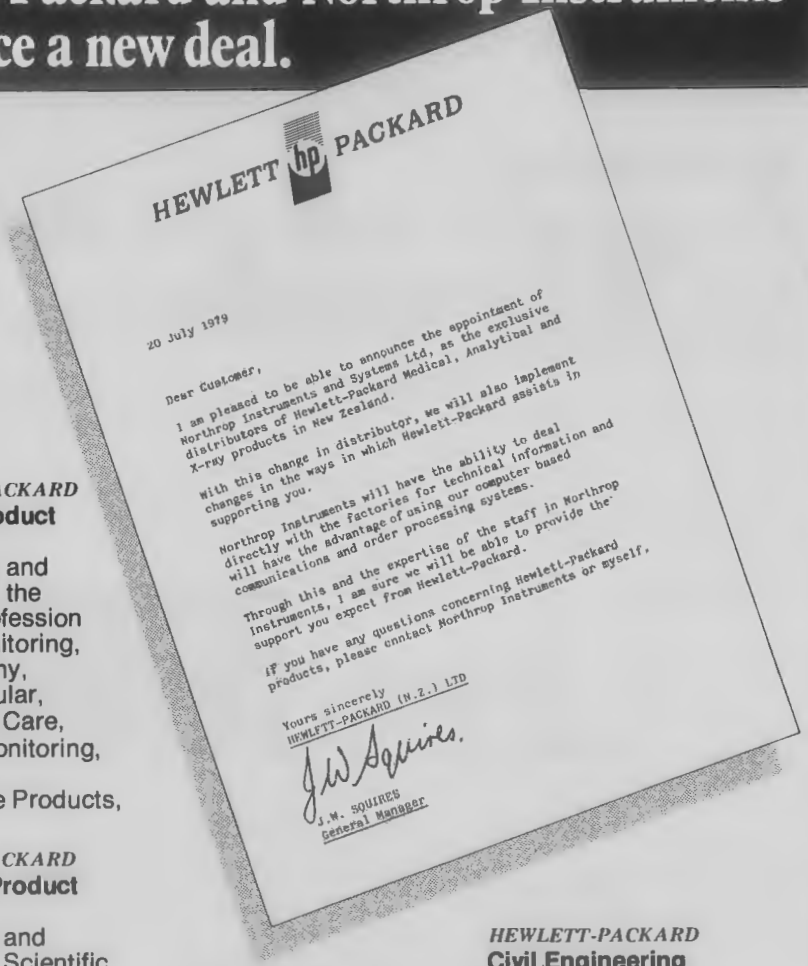

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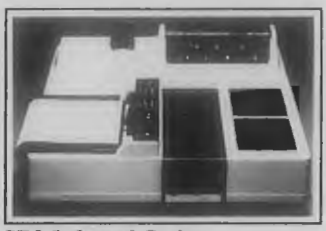

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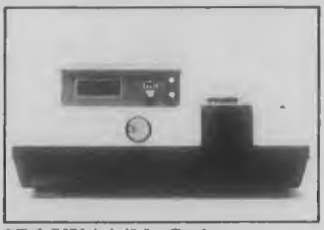

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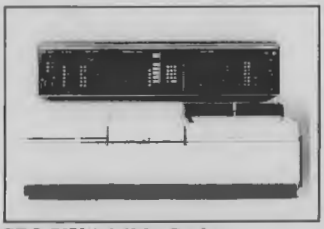

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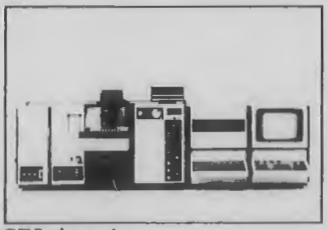

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avidity is observed. These same authors are already finding LISS's value in the microelution technique which they use for the detection of antigens in blood stains where so often the antigen as well as being severely limited in amount, is impaired through drying.

Issitt, *et al.* (1978)[5] have shown that most IgG non-agglutinating antibodies give enhanced reactions in LISS, many of them become capable of causing agglutination of red cells prior to the Indirect Coombs Test. A few IgG and some IgM antibodies fail to give enhanced reactions in LISS, perhaps because of partial precipitation of these antibodies at low ionic strength. These authors have shown that Lewis antibodies appear to be the only antibodies that on even rare occasions give better reactions in saline or albumin than in LISS.

When Standard A and B cells suspended in LISS were used in our laboratory for reverse ABO grouping of patients and donors almost all positive results showed haemolysis as well as agglutination after one hours incubation at Room Temperature or 37 °C demonstrating the increased uptake of complement components at low ionic strength. The importance of this is that LISS should not be used as the suspending medium for cells for reverse grouping of donors, as the number of donors with haemolysins detected would be artificially high. Although the recommended incubation time for techniques using LISS suspended cells is 10-15m and our investigations have shown there to be little or no haemolysis within that time, unless extra care is taken in looking for haemolysis then there could be a danger, when crossmatching, of missing a clinically significant haemolytic antibody.

The use of LISS is clearly indicated when only small amounts of patient's serum are available because one drop of the patient's serum can be used in the sensitive test system. For the same reason LISS will be of value in working with rare antisera or where a large number of tests must be performed with a limited amount of serum. Issitt *et al.* (1978)[5].

Conclusion

LISS has proven to be a rapid, simple and sensitive technique for the detection of blood group antibodies. With a total of 12 years experience of LISS and to date no significant problems associated with its use it would appear that LISS will continue to be a useful tool in the Immunohaematologists armoury of *in vitro* test methods.

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A Blood Group Survey of New Zealand Resident Cook Islanders

Jenny Daniels, ANZIMLT, AIMLS and C. G. Storey, ANZIMLT
Pathology Department, Tokoroa Hospital
and Immunohaematology Department, Waikato Hospital
Accepted for publication June 1979

Summary

Blood groupings were carried out on 80 Cook Islanders, resident in the Tokoroa area. As in other Polynesian studies, No. A_2 or rr individuals were found. No Kell positive people were found and SS individuals were rare. The blood group frequencies from this survey were compared to previously reported data from New Zealand Maori and European populations. Similarities to both groups were found.

Introduction

A haematology survey had been carried out at Tokoroa, and the 80 Cook Islander samples used for this blood group survey were obtained from the 300 general population samples, collected for the original survey. The fact that some members of the population were related cannot be excluded. In a small number of samples such as this the relationship may influence the results. The blood was collected into ethylene-diamine tetra-acetic acid, and the groupings carried out within a few days of collection. The systems investigated were ABO, MNSs Kell, P. Duffy, and Rhesus.

Methods

ABO and Rhesus Phenotypes were performed on a Bg 8 autoanalyser. The BG 8 system utilises bromelin and polyvinyl pyrrolidone to enhance blood group reactions, and the system remains at room temperature throughout. ABO typing utilised anti-A, anti-B, anti-A + B and anti A_1 (obtained from *Dolichos biflorus*) and reverse grouping included O, A_1 and B cells. Rhesus genotyping involved the use of anti-D, anti-C, anti-c, anti-E, anti-e. The main advantage of using the BG8 was the small volumes of anti sera needed.

M, N, and P_1, typings were carried out by room temperature sedimentation methods for one hour. Kell, Fy^a, Fy^b, S and s, typings were obtained by the indirect Coombs test after incubation at 37 °C for one hour. Smith-Biolab broad spectrum Coombs reagent was used for the indirect Coombs tests.

The blood group anti-sera were obtained from various sources including Dade, Ortho, and Smith-Biolab and some were made by the Waikato Blood Transfusion Services.

Results

Blood Group Frequencies within the Cook Island Population

ABO System

O = 33%	A_1 B = 0%	B = 10%
A_1 = 57%	A_2 = 0%	

MNSs System

M positive = 75%	N positive = 69%
MM = 31%	NN = 25% MN = 44%
S positive = 10%	ss = 90%

Only two samples were found to be s negative, i.e., only 2.5% of the population were SS.

P System

P_1 positive = 52%.

The P_1 antigen was showing variation in strength of expression as it does in other populations. Positive results varied from + to 4+ reactions.

Kell System

No Kell positive individuals found.

Duffy System

Fy^a+ = 87%	Fy^b+ = 48%
Fy^a+^b- = 52%	Fy^a+^b+ = 35%
No Fy^a-^b- individuals were found	Fy^a-^b+ = 13%

Rhesus System

R_1R_2 = 54%	R_1R_1 = 22% R_2R_2 = 16%
rr = 0%	R_1R_0 = 8%

Discussion

The percentages shown above were compared to those relating to NZ Maoris[5] and Europeans [1,4]. The results are shown in graph forms in figures 1-3. The results of the MNS and ABO systems agree reasonably well with those of R. Douglas and J. M. Staveley in 1959[3]. The results of the Duffy and P systems compare favourably with the work done on Atiu Islanders by R. Douglas et al[2].

ABO System (figure 1)

There are more group A in the Cook Islanders than in European or New Zealand Maoris, although as in other Polynesians the A_2 gene appears to be absent. Group B appears to occur in similar proportions in Europeans and Cook Islanders, although in other Polynesian studies group B is rare, and in fact is absent in the NZ Maori. However, it is reported as present in Samoans and other populations with Melanesian origins.

MNS System (figures 2 and 3)

The frequencies for M and N groups are similar in Europeans and Cook Islanders, but reversed in the NZ Maori. That is, there are more N positives than M positives in the NZ Maori, where as in the Cook Islanders and the Europeans there are slightly more M positives than N positives.

The S gene is infrequent in the Cook Islander as it is in the NZ Maori. Only 2.5 percent of Cook Islanders are SS compared to 11 percent of Northern Europeans.

P System (figure 3)

P_1 negative individuals are more frequently found in Polynesians than Europeans. The figures obtained for Cook Islanders in this survey fit with these findings. Tokoroa has a high Polynesian population which may be a contributory factor to the fact that 17% of the irregular antibodies found in this laboratory are anti-P_1 in specifcity.

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COMPARISON OF BLOOD GROUP FREQUENCIES WITHIN COOK ISLANDER EUROPEAN AND NEW ZEALAND MAORI POPULATIONS.

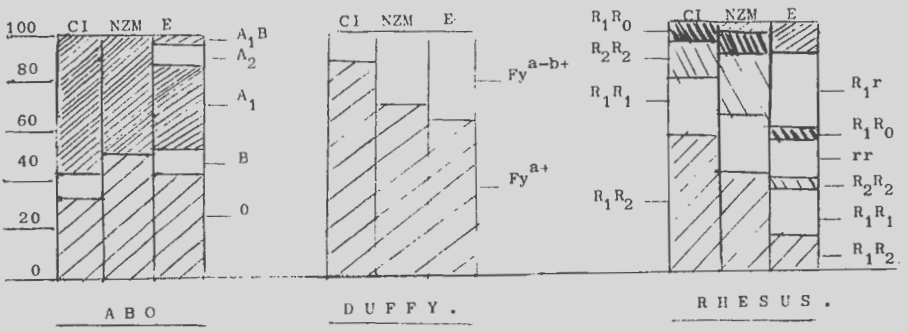
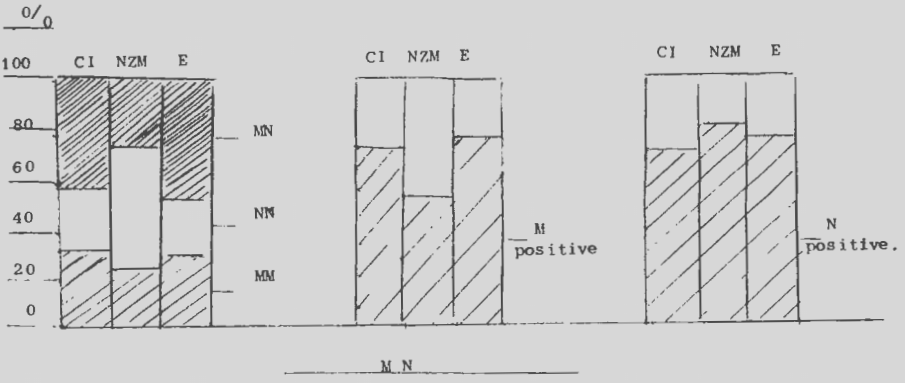


figure 1.



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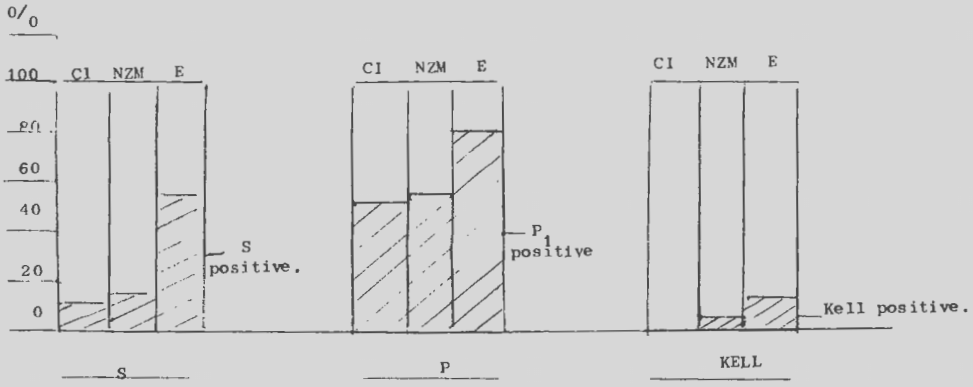

figure 3.

Kell System (figure 3)

No Kell positive individuals were found in this survey. However, a larger number of samples may have resulted in a few Kell positives. For comparison 2% of NZ Maoris are Kell positive, but a study of 237 people was needed to arrive at this figure[5].

Duffy System (figure 1)

Fy^a-^b+ individuals occur far less frequently in the Cook Islanders than in the NZ Maori or European. Fy^a+ people are more frequently found and no Fy^a-^b- types were found.

Rhesus System (figure 1)

The rr genotype was not encountered in the Cook Islanders as it is not in other Polynesian groups. R_1R_2 was the most frequently occurring genotype in the Cook Islanders as it was in the NZ Maori. The phenotype Cc Dee is most likely to represent the genotype R_1R_0 in Polynesians rather than R_1r as in Europeans.

Conclusion

Although Polynesian blood groups are now well documented, it was thought that a short comparison of blood group frequencies within New Zealand populations might be of interest to students and other technologists recently gaining an interest in the subjects related to a Blood Bank.

Acknowledgments

We wish to convey our thanks to the Waikato and Tokoroa Hospitals for the use of their laboratory facilities for this survey, and to Dr T. Caradoc-Davies for arranging the sample collection.

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Suitability of Commonly Used Enrichment Broths and Diluents For Enumerating *Vibrio Parahaemolyticus*—A Review of Current Literature

Barbara J. Hayden, B. Tech (Hons)

National Health Institute, P.O. Box 7126, Wellington South

Received for publication, March 1979

Summary

Vibrio parahaemolyticus cells are very sensitive to temperature and salt concentration especially if they have been sublethally damaged as is often the case in processed foodstuffs. A number of diluents and enrichments broths commonly used for the enumeration of *V. parahaemolyticus* are actually deleterious to the organism. 0.1M potassium phosphate buffer with 3 percent NaCl appears to be the most suitable diluent. Sublethally injured cells may require resuscitation in a low-salt environment before being subjected to a high-salt enrichment broth. It is recommended that two different enrichment broths be used in parallel.

Introduction

A number of diluents are used for the enumeration of *Vibrio parahaemolyticus* in foods. Many laboratories use the same diluent as for the enumeration of other food pathogens. However, this may not be the most suitable diluent to use since *V. parahaemolyticus* is very sensitive to temperature and salt concentration especially if there has been sub-lethal damage to the cells during processing of the food.

Glucose salt teepol broth (GSTB) is the commonly used enrichment broth since it is recommended by the US Food and Drug Administration and the American Public Health Association. However, a number of workers have found other broths which are superior to GSTB in their ability to resuscitate and support the growth of *V. parahaemolyticus.*

Diluents

V. parahaemolyticus is isolated frequently from coastal waters and seafoods from all parts of the world and is the most frequent cause of foodborne disease in Japan, where many residents eat raw fish. In New Zealand there has been one confirmed outbreak of food poisoning caused by *V. parahaemolyticus* in raw mussels, Dove (1978)[5].

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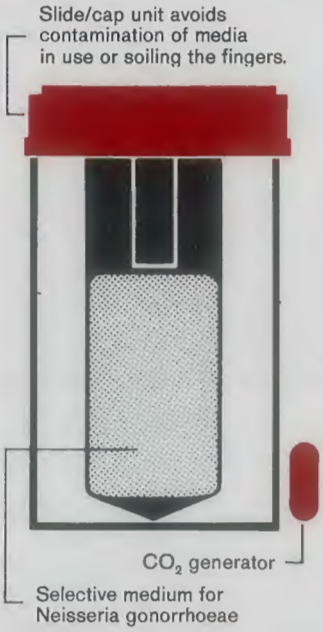

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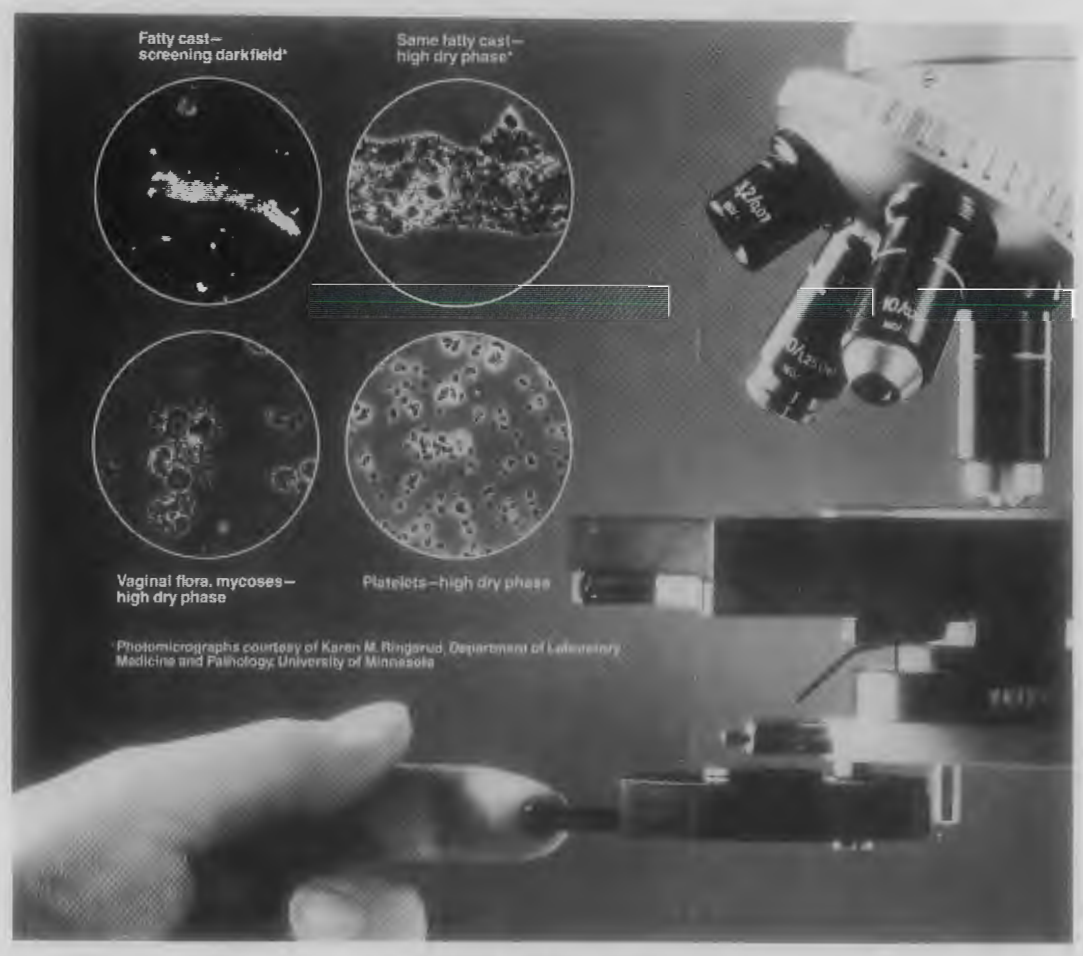

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hibit injury on exposure to freezing and chilling environments. These cells are more sensitive than are non-injured cells to selective conditions in most enrichment broths. It has been demonstrated that cells of *V. parahaemolyticus* exhibit injury when chilled at 2°C for as little as 30 minutes, Beuchat (1977)[2]. Other workers have reported that *V. parahaemolyticus* is readily inactivated at refrigeration and freezing temperatures (4, 8, 10). Various inactivation rates have been reported, depending on the type of seafood examined and the media used for detection and enumeration.

This raises questions concerning the suitability of dilution fluids such as water, saline, peptone, and phosphate buffer, commonly used in making bacterial counts.

Emswiler and Pierson (1977)[6] found that 100mM potassium phosphate buffers at various pH without added NaCl were extremely deleterious to *V. parahaemolyticus.* Maximum counts were obtained when phosphate buffers containing 3 percent NaCl were used as diluents. Greater than 99.9 percent of the cell population was destroyed within 20 minutes in 0.1 percent peptone diluent without NaCl when compared to 0.1 percent peptone with three or 6 percent NaCl. These results are not surprising since *V. parahaemolyticus* is a marine organism and requires NaCl for growth and viability. Survival data for *V. parahaemolyticus* in distilled water containing added salt were similar to data for the 0.1 percent peptone diluents.

Beuchat, (1977)[2] also concluded that 3 percent NaCl in 0.1M potassium phosphate (pH 7.0) was the best diluent to prevent inactivation of cold—and heat-injured cells.

Enrichment Broths

In the same study, Beuchat looked at the effect of a number of enrichment broths on sublethally injured *V. parahaemolyticus* cells and found that exposure of chill-injured *V. parahaemolyticus* to GSTB and trypticase soy broth with 7 percent salt (TSBS-7) resulted in 70-80 percent death; about 70 percent lethality was noted for heat-injured cells inoculated in TSBS-7. GSTB did not appear to be lethal to heat-injured cells. For both types of injury, Horie arabinose ethyl-violet broth (HAEB) and water blue alizarin yellow broth (WBAY) appear to be superior to GSTB for supporting repair and subsequent division of cells, although rates of growth were retarded.

For the most probable number (MPN) method, HAEB, salt peptone broth with 3 percent NaCl and 1 percent peptone (SPB) and WBAY media were all superior to GSTB, the enrichment medium recommended for isolation of *V. parahaemolyticus* from food samples, and to TSBS, an alternative enrichment medium, Beuchat, (1976)[1].

SPB is non-selective and would seem to have limited application in the isolation of *V. parahaemolyticus* from foods harbouring a mixed microflora. Later studies by Beuchat (1977)[3] have revealed that many false-positives were noted when WBAY was used as an enrichment broth probably because of its relatively non-selective pH (6.9).

However, it appears that HAEB and WBAY merit inclusion in schemes being examined for the routine enrichment and isolation of *V. parahaemolyticus.*

In a survey of shellfish and estuarine waters carried out in the Netherlands, Kampelmacher *et al* (1972)[7], found it necessary to use at least two different enrichment procedures as well as isolation methods in parallel to obtain reliable results. This is a situation similar to that in the detection of salmonellae in various food products and so is not exceptional.

Incubation Time

Culture age and time of exposure to the diluents also significantly affected survival of *V. parahaemolyticus.* Ray et al (9) inoculated *V. parahaemolyticus* into GSTB and trypticase soy broth with 3 percent (TSBS-3) to establish growth rates and found that the cells reached the stationary phase of growth in TSBS-3 within 10-12 hours and then began to die off. In GSTB, the cells reached the stationary phase by eight hours and then began to die off. The results of this study indicate that a low population of uninjured cells of *V. parahaemolyticus* can multiply and reach their maximum population level within 8-10 hours of growth in a selective (GSTB) and a non-selective (TSBS-3) broth. However, because the cells show sensitivity to GSTB, the sample should be tested at or before eight hours to obtain higher detection.

Discussion

The sensitivity of several strains of *V. parahaemolyticus* cells to refrigeration and frozen storage has been observed by a number of workers (2, 4, 8, 10). Cell death occurs at a very rapid rate under both storage conditions, and 90 percent or more of the survivors may be injured Ray *et al.* (1978)[9]. Refrigeration temperatures appear to be more lethal than frozen storage. Detection methods must be capable of resuscitating the injured cells as well as supporting the growth of the organism.

Ray *et al.* (1978)[9] recommend that sublethally

injured cells be resuscitated in a low-salt environment (0.5 percent NaCl in TSB) for one to two hours at 35 °C. Once the cells have passed the "repair phase" and entered into the "multiplication phase," they are exposed to a high-salt environment such as TSBS-3 at 35 °C overnight. Next morning, the sample is transferred to GSTB for selective enrichment and incubated at 35 °C for 6-8 hours before streaking on to thiosulphate citrate bile salts sucrose agar (TCBS).

This procedure can be easily fitted into an 8-hour working schedule. As a precaution, all GSTB could be reincubated overnight and restreaked next morning if no *V. parahaemolyticus* had grown on the 6-hour plates.

It is also recommended that a second enrichment broth such as HAEB be inoculated in parallel with the GSTB.

The most suitable diluent appears to be 0.1M potassium phosphate buffer with 3 percent NaCl.

Acknowledgments

I am indebted to the Director-General of Health, Department of Health, Wellington, for permission to publish this paper.

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Technical Communications

An Unusual Isolate

A 26-year-old male presented with a typical pilonidal sinus which had been infected for four days. The sinus was already discharging and no draining was required. We received a swab, from the discharging sinus, in Stuarts Transport medium with charcoal. The swab was immediately cultured to Blood and MacConkey agar plates and the swab placed in Thioglycollate broth containing Vitamin K1 and haemin. After incubation at 37 °C in 10 percent CO_2 for 18h a moderate growth of a gram negative bacillus was noted on the plates. The organism was identified as an *Acinetobacter* species by the API system.

A gram stained smear of the broth revealed a small gram negative cocco-bacillus. Suspecting an anaerobic organism, the broth was subcultured to fresh anaerobic culture media—Blood agar and Vancomycin-Kanamycin laked blood[3] plus aerobic blood agar. The anaerobic plates were incubated at 37 °C in an anaerobic jar with a Gaspak. After 48h a very small greyish colony was noted on the anaerobic blood only. The gram stain of the colony revealed a small gram negative bacillus with some pleomorphic forms. A subculture of the colony was made into thioglycollate broth and incubated for 4h. An API 20A strip was inoculated from the broth, incubated in an anaerobic jar overnight with sensitivities being performed at the same time. From the gram stain and colony form we were expecting results identifying the organism as a *Bacteroides* species. However, results obtained failed to clearly elucidate the organism.

In order to avoid delay in patient therapy a preliminary report was sent out, giving sensitivities of the anaerobic organism as well as the Acinetobacter.

Dr G. Tannock, Microbiology Department; Otago University, was then consulted regarding the anaerobe. After several subcultures he decided the organism was in fact a gram negative coccus. Identification using gas liquid chromatography was then performed. It was found that the organism produced mainly acetic and butyric acids from fermentation of glutamine and propionic and butyric acids from threonine. It did not utilise carbohydrates or lactate. On these results the organism was identified as *Acidaminococcus fermentans.*

Bergey[1] describes this species as the only member of the genus Acidaminococcus in the Family Veillonellaceae. Bergey states it is probably non-pathogenic, as isolates have been obtained from the gut of normal animals—pigs and man. One authority does record it in a human clinical isolate[2].

Whether this isolate was pathogenic in this in-

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stance is uncertain, however it was isolated from an infective process. The patient was treated with Orbenin with no further problems.

My thanks go to Dr D. Cook for providing clinical details and Dr G. Tannock for his study of the organism and the information he provided.

John Finalyson,
Doctor's D. J. Perry,
N. W. Fitzgerald,
Medical Laboratory,
Dunedin.

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L. casei Folate Assay

For over 10 years this laboratory has measured folates by the *L. casei* assay using commercial media. No difficulty was experienced with this assay until about two years ago, but since that time we have found it impossible to obtain consistent results. We use *L. casei* NCIB 8010 and Difco Folic Acid Casei Assay Medium (Control Number 635971), and carry out the assay according to standard techniques.''[2]

The difficulties experienced were due to poor growth in the tubes of the standard curve which contained the added folic acid. The assay tubes containing serum and red cell extracts grew normally. This resulted in the observed folate levels being erroneously high. The readings of the standards varied considerably from day to day. The highest standard, equivalent to a serum of 32μg/1, could read anything from 0.15 OD to 0.5 OD units.

We have corrected this fault by lowering the pH of the media to pH 6.0 with lactic acid. This stabilises the pH during subsequent incubation at 37 °C for both standards and specimen extracts. During incubation, the pH of the unaltered media

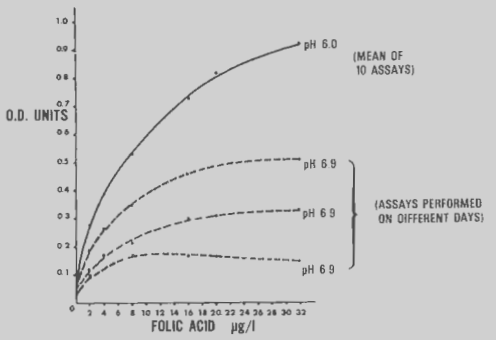

Figure 1.—Folic acid standard curves showing the effect of lowering the pH of the medium to pH 6.0 by adding lactic acid.

containing the standards fall to a different pH to that of the media containing the specimen extracts. The above alteration gives a consistently better standard curve with the top standard reading about 0.9 OD units (see figure 1). The majority of the samples being assayed then give optical densities which fall within the range of the standard curve. Having altered the media, we checked for recovery and reproducibility. These were both satisfactory. Following this modification the observed normal range for serum and red cell folate showed good correlation with a folate radioassay. (Amersham radio-folate kit, Radiochemical Centre, Amersham, Buckinghamshire, England).

While there may be batches of commercial *L. casei* assay medium already at optimal pH, it is suggested that each new batch be tested and if necessary modified as described above.

J. Tizzard,
Department of Haematology,
Christchurch Hospital,
Christchurch.
June, 1979.

REFERENCES

1. **Hoffbrand, A. V., Newcombe, B. F. A. and Mollin, D. L. (1966),** *J. clin. Path.* **19,** 17.
2. Waters, A. H. and Mollin, D. L. (1961), *J. clin. Path,* **14,** 335.

Abstracts of Papers read at a Scientific Meeting of the Canterbury Branch of the NZACB, August 1978.

Structural and Functional Abnormality of Pro-albumin, Christchurch. S. O. Brennan, Department of Clinical Biochemistry, Christchurch Hospital.

A slow migrating albumin variant, representing 50 percent of the total albumin, was found in four generations of a Christchurch family. Investigation of this bisalbuminemia by sequence analysis

of the intact protein and of tryptic and *S. aureus* V8 protease peptides, showed the presence of six additional amino acids at the N-terminus of the variant. The extra amino acids, Arg-Gly-Val-Phe-Arg-Gln-, are analogues to those found in rat and bovine proalbumin which contain the additional N-terminal sequence of Arg-Gly-Val-Phe-Arg-Arg-.

This variant has been named proalbumin Christchurch and demonstrates for the first time the sequence of human proalbumin. A single base change n the DNA coding for human proalbumin would result in the mutation Arg-Gln thereby preventing the cleavage of the propeptide before secretion from the hepatocyte.

While there is no obvious clinical condition associated with this abnormality spectral and dialysis studies show that the high affinity Cu(II) binding site is blocked in this variant. A simple electophoretic test has been developed utilising Ni63(II), which allows the discrimination of this from other albumin variants.

The effects of limited tryptic digestion of proalbumin Christchurch suggest that trypsin is not the *in vivo* enzyme responsible for the conversion of proalbumin to albumin.

Two Radioimmunoassay Methods for Measuring Plasma Oestriol. J. J. Evans, University Department of Obstetrics and Gynaecology, Christchurch Women's Hospital, Christchurch.

Antisera were raised in rabbits against 6-ketooestriol 6-carboxymethyloxime: BSA. The antisera were specific to oestriol and did not react significantly with oestriol isomers or with other oestrogens as detected by radio-immunoassay. However, the antisera reacted with oestriol conjugates. The antisera were used in the investigation of two radioimmunoassay methods for measuring plasma oestriol. the first method assayed plasma directly without extraction or separation steps. It was found that when the direct method was used different antisera estimated different immunoreactive oestriol levels in the same plasma sample. It appeared that the dependency of the immunoreactive oestriol concentration on the antiserum employed was due to the antisera reacting to different extents with the same oestriol conjugate. The second radioimmunoassay method involved extracting plasma samples with ether before assay. Conjugates were not extracted in detectable concentrations by the ether. When plasma samples were assayed by the second method the results obtained were independent of the antiserum used. The introduction of an ether extraction step resulted, therefore, in a more suitable radioimmunoassay method for monitoring plasma oestriol concentrations.

Some Pitfalls in the Quality Control of Alkaline Phosphatase Activity. R. Fowler, Department of Clinical Biochemistry, Christchurch Hospital.

The validity of serum standardisation of multichannel analysers is based upon the assumption that the proportional response of the calibration serum to that of the patient specimens is constant, within small changes in reaction conditions. A wide variation in the pH optimum of alkaline phosphatase activity in various calibration and control serum preparations exists, and as a consequence, as small an error of ± 0.05 pH units in the preparation of the buffered substrate results in an error in the order of ± 5 percent in the proportional response between the calibrating serum and patient specimens.

It is recommended that a calibration serum should closely parallel the pH optimum of alkaline phosphatase in human sera and that a quality control serum containing alkaline phosphatase of alternative source to that present in the calibration serum be included with patient samples as a sensitive means of monitoring pH changes in the buffered substrate.

α-1-Antitrypsin Phenotyping Using Agarose Gel. Merle Sheat and Sue McQuilkan, Department of Clinical Biochemistry, Christchurch Hospital.

α-1-Antitrypsin is a glycoprotein which inhibits the action of proteolytic enzymes including trypsin, thrombin, plasmin and elastase in the serum. It is under the genetic control of a pair of codominant autosomal alleles at a single locus, Pi (Protease Inhibitor). At least 23 electrophoretic variants of α-1-antitrypsin have been identified and designated alphabetically in order of migration at pH 5.0. Deficiencies in α-1-antitrypsin may be associated with emphysema and liver cirrhosis.

A new method for phenotyping α-1-antitrypsin is described. Initially α-1-antitrypsin was phenotyped using acid starch gel electrophoresis followed by crossed immuno-electrophoresis into agarose containing α-1-antitrypsin antisera. As phenotyping by this method is both a time-consuming and technically difficult procedure, a new 2-dimensional technique using agarose gel was developed.

Proteins are separated in the first dimension in agarose gel at pH 5.0 using a sodium acetate acetic acid buffer. The pH of the buffer is critical. The α-1-globulin fraction is then crossed into agarose

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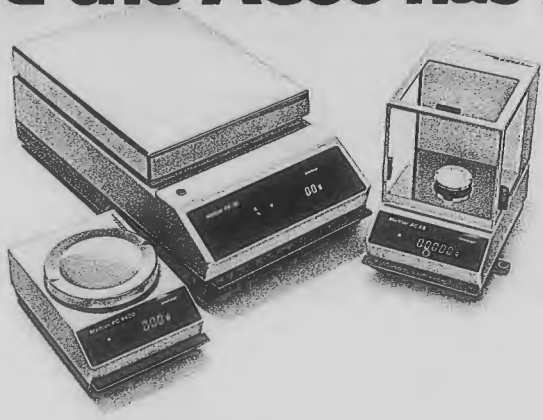

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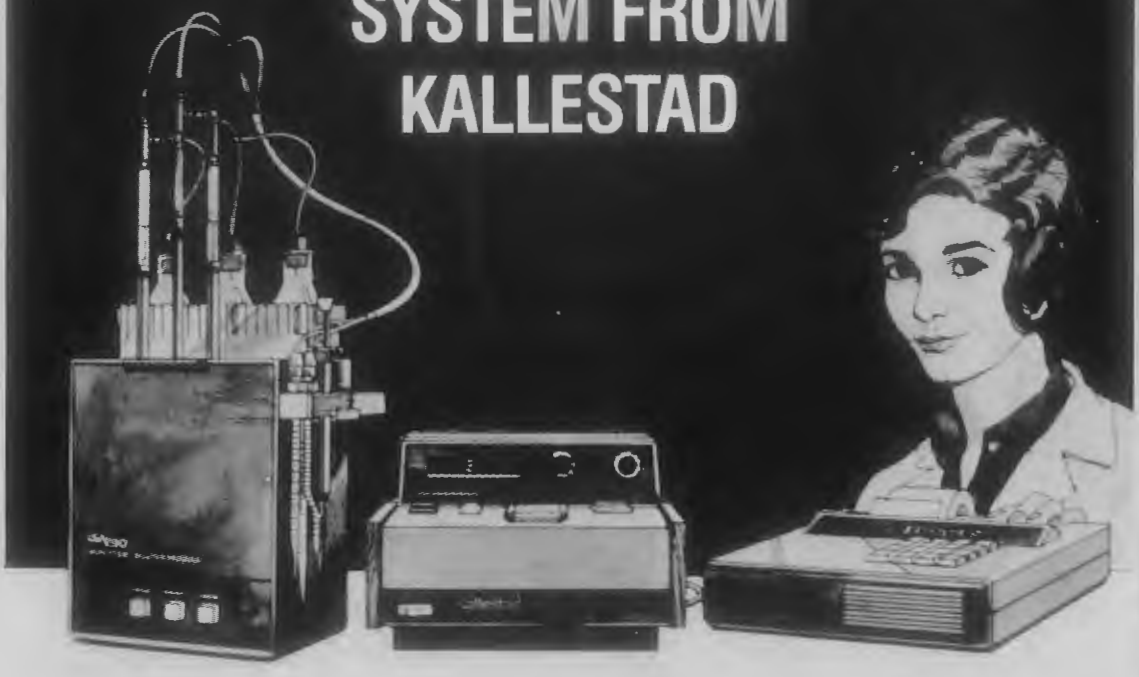

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Using the techniques just described 410 male blood donors were phenotyped resulting in a percent frequency for MM of 87.3 percent. The percent frequencies of other α-1-antitrypsin phenotypes also compared favourably with the percent frequency of 1.0 percent obtained for the MZ phenotype is comparable to that of Great Britain—1.5 percent, Spain—1.9 percent and Netherlands—1.0 percent. However, a frequency of 4.7 percent for the MZ phenotype has been reported for the Norwegian population.

α-1-Antitrypsin quantitation was carried out on 307 male blood donors resulting in a normal range of 1.3-3.2g/l with a mean of 2.3g/l.

Studies of Tamm-Horsfall Urinary Glycoprotein. P. R. Macdonald, J. S. Hunt and A. R. McGiven, Department of Pathology, University of Otago, Christchurch Clinical School of Medicine.

Tamm-Horsfall urinary glycoprotein (THP), prepared by salt precipitation of pooled urine from normal donors, has been shown to stimulate human peripheral blood lymphocytes (PBL) to undergo blastoid transformation. Several preparations of THP stimulated, in varying degrees, all the PBL's tested. Stimulation was measured by the uptake of tritiated thymidine into DNA. The response in four day cultures approached that seen with several mitogens and the response in six day cultures usually exceeded that seen with tuberculin purified protein derivative (PPD) with Mantoux positive lymphocyte donors.

Studies of the biochemical and immunological properties of THP were undertaken to compare stimulatory with non stimulatory preparations. Results from these studies have shown that:

THP contains one major antigenic component (by crossed immuno-electrophoresis); has a native MW > 1 x 10^6; has a subunit MW ~ 100,000; contains ~ 23 percent carbohydrate; is composed of a fibrillar chain in a zig-zag configuration 25 A x 100 A, as confirmed by electron microscopy.

The function(s) of THP still remain obscure, but recently cell mediated immunity and lymphocyte cytotoxicity against it have been reported in active chronic hepatitis and primary biliary cirrhosis associated with renal tubular acidosis. This could suggest a pathological association of THP with some renal diseases and this possibility is presently being studied in animal models.

"Surface Tension" Effects in the Analysis of Plasma Creatinines by Continuous Flow Analysis. T. A. Walmsley, Department of Clinical Biochemistry, Christchurch Hospital.

In the analysis of plasma creatinines by continuous flow analysis it was observed that the last drift control in a batch of specimens was always higher than the previous drift controls. This was not a gradual drift upwards within the batch of specimens but a sudden jump in the result of the last drift control.

When the first specimen is in the dialyser, the membrane sags, this causes the flow rate of the recipient stream from the dialyser to momentarily speed up; this results in over-dilution of the picric acid reagent and a dip occurs in the baseline before the first peak. After all the specimens have passed through the dialyser the membrane relaxes back to its original position—thus the recipient stream would momentarily slow down; this would result in the picric acid being underdiluted and cause the absorbance of the last peak to be higher than expected.

The pressure across the dialyser membrane increases when a specimen passes through and the pressure difference can be minimised by increasing the detergent concentration in the donor stream of the dialyser.

***In-Vitro* Stability of Endogenous Peptide Hormones in Plasma and Urine.** J. H. Livesey, Medical Unit, Princess Margaret Hospital, Christchurch.

This study was performed to determine whether samples for radioimmunoassay could be transported to the laboratory at ambient temperature or in ice, as an alternative to being sent frozen.

Fresh human plasma samples (1mg EDTA/ml) were incubated in polystyrene tubes for 1 to 8 days (d) at 4°, 20°, 20° and 37°. Controls were stored at -20°. The changes in immunoreactivity were measured by radioimmunoassay for the following endogenous hormones: insulin, glucagon, prolactin (HPrL), growth hormone (HGH), luteinising hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). The stability of LH and FSH in urine was similarly studied. The effect on immunoreactivity of up to five times repeating freezing at -20° and thawing to +20° was also investigated for glucagon, LH, FSH, TSH and HGH in plasma.

Insulin immunoreactivity (number of samples from different subjects = 5) showed no significant change (<20 percent) after 8d at 4°, three

samples showed significant loss (> 20 percent) after 8d and 20° and all samples showed significant loss after 2d at 37°.

Glucagon immunoreactivity (n = 2) showed no significant loss after 1d at 4° or 20°, but after 8d at 4°, 2d at 20° or 1d at 37° losses were significant.

Prolactin immunoreactivity decreased significantly in one of three samples after 8d at 4°, in three or four samples after 8d at 20° and in four of five samples after 8d at 37°.

Growth hormone immunoreactivity was not significantly changed after 8d at 4° (n = 5), two of seven samples showed significant loss after 8d at 20°, as did two of six samples after 4d at 37°.

LH immunoreactivity (n = 4) was not significantly altered after 8d at 4° or 20°, but two samples showed significant loss after 8d at 37°.

FSH immunoreactivity (n = 4) was not significantly changed after 8d at 4°, one sample showed a significant loss after 8d at 20° and two samples showed a significant loss after 8d at 37°.

TSH immunoreactivity (n = 2) showed no significant loss after 8d at 4° or 20° but two samples showed a significant loss after 8d at 37°.

Urinary LH (n = 2) and urinary FSH (n = 2) showed no significant decrease after 8d at 37°.

Up to five times repeated freezing and thawing of plasma had no significant effect on levels of glucagon (n = 2),, LH (n = 2), TSH (n = 1) and HGH (n = 2). One of two samples showed a significant increase in FSH levels.

It is concluded:

(i) That insulin, HGH, HPrL, LH, FSH and TSH but not glucagon plasma samples could safely be transported to the laboratory in ice as an alternative to deep frozen transport.

(ii) That HGH, LH, FSH and TSH samples could be consigned at ambient temperatures by airfreight or post, at least in temperate regions.

Drug Analysis by Electron Capture—GLC. Optimization of Response. T. Lindley, Department of Chemical Biochemistry, Christchurch Hospital.

The temperature dependence of electron capture coefficients has been known for over 10 years, however, until the arrival of the Ni^{63} detector, stable to 400°, this property could not be readily investigated.

Two classes of mechanisms have been described for electron capture processes, and they exhibit opposite temperature dependencies (1, 2, 3). In dissociative electron capture the rate determining step of the overall process is one in which a bond is broken, therefore higher temperatures favour the process, and the detector response increases with temperature. In non-dissociative electron capture the anion formed on electron capture is stable, and the reverse of electron capture, namely loss of an electron from the anion to produce the original molecule, is significant. As the anion is of lower energy, the reverse process becomes more competitive at higher temperatures, and the response decreases.

For both electron capture mechanisms a plot of $\ln KT^{3}/2 vs {}^{1}/T$ where K is the capture coefficient, will be linear (1). For dissociative electron capture the slope is negative, and for non-dissociative electron capture, positive. Both mechanisms may have a temperature region where the rate of electron capture is determined by other factors such as the rate at which the anion reacts with positive species, and the temperature dependence is negligible.

We have studied the temperature dependence of electron capture detector responses of three drugs commonly analysed in plasma extracts by electron capture—GLC, and the internal standards used in these analyses in our laboratory.

Chlorpromazine and clomipramine, both produced strongly negative slopes on plotting $\ln KT^{3}/2$ vs ${}^{1}/T$ over the range 250° to 420°, with peak heights at the maximum temperature being over an order of magnitude higher at the top of the range. This indicates that both these drugs capture electrons dissociatively presumably by cleavage of the halogen from the aromatic ring to form a halide anion and an aromatic radical.

Diazepam and flurazepam both produced positively sloped plots, with regions of zero slope below 280°, consistent with capture by a non-dissociative mechanism. In benzodiazepines, the molecular structure allows for resonance stabilization of the anion, which is not as readily available in the tricyclics, chlorpromazine and clomipramine.

The ethyl derivatives of clonazepam and desmethylflu-nitrazepam showed little temperature dependence in the electron capture detector, but by analogy with diazepam, probably capture electrons non-dissociatively, the reverse reaction not yet being rapid enough to compete with loss of the anion by reaction with positive species.

In conclusion, highest detector responses occur at high temperatures for chlorpromazine and clomipramine, and at low temperatures for diazepam and flurazepam. As the background of coextractives present in analytical samples

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Charles Sydney Shepherd

Born:	Te Puke 18 January, 1934.
Educated:	Correspondence School, Paengaroa Primary, Te Puke High. School Prefect and Dux.
1952	Ardmore Training College and did part-time Auckland University (got Education, Psychology).
1953	Commenced Thames Hospital Laboratory as Trainee under Ian D. Scott.
1956	Passed Basic Training Certificate.
1957	Commenced Waikato Hospital Laboratory.
1958	Married in June, passed C.O.P. in August (highest mark in Chemistry, lowest was Haematology!).
1960	Commenced Hamilton Medical Laboratory in charge of Haematology (staff of 3)—still there.
1967	Elected to NZIMLT Council on formation of CNI region. Remained till 1973.
1971 & 1973	Short study trips to United States.
1973	Vice President NZIMLT, appointed to new Medical Technologists Board.
1975	Smith Biolab Travel Award.
1976	Deputy Chairman MTB.
1978	President NZIMLT.
1979	Re-elected Deputy Chairman MTB.

Committee Assignments: (at some time). NZIMLT—Membership, Education, Registration. MTB—Registration, education, Examinations. Examiner (Haematology) BTC, Part II, Part III 1970-78. Married with two girls, three boys. Wife is a Registered Nurse (practicing).

Interests: *Sports*—Athletics in earlier years, now some spasmodic coaching to a few enthusiasts. Rugby—enjoyed playing, did some coaching, now a supporter. Basketball—supporter of sons and daughter who play. *Church Work*—Youth work, counselling, teaching, leadership training, scouting (have my woodbeads). *Other*—Territorial army many years—reached Major. *Music*—have sung in choirs, quartets (used to tootle on clarinet years ago). *Art*—not abstract! Reading and poetry. *Tramping, exploring*—though not much time these days!

Find most of my spare time (and money) is taken up now with a growing, energetic, ever-hungry, sport-loving family.

Ivor W. Saunders 1902-1978

G. D. C. Meads

Laboratory, Taranaki Base Hospital

The NZIMLT Council is conscious of the need to record any available information about the origin and development of medical laboratory technology in New Zealand and is grateful to Mr Meads for fossicking around and coming up with these interesting details of the late Ivor Saunders, one of our founder members. This article is a useful addition to our archives as it shows the difficulties experienced in the early days when medical technology was developing, set against a background of economic difficulty and natural catastrophe.

Editor.

Ivor Saunders was born in 1902 at Wadestown, Wellington. For the next four years his father was a partner in a saw milling business until the family moved to Ngaere, Taranaki to take up farming. Here the young Ivor had to help with the usual work expected of a farmer's son and in those days included helping in the cowshed, clearing the land of stumps, rushes, blackberry, and cleaning drains. He attended the Ngaere school where he distinguished himself by winning a Junior National Scholarship (now abolished), which entitled him to a place at a secondary school together with an annual monetary allowance. After some years at the Stratford District High School where he acquired that love of literature, especially poetry, which he retained throughout his life, he was appointed to a Cadetship in the Department of Health, Wellington and attended lectures at Vic-

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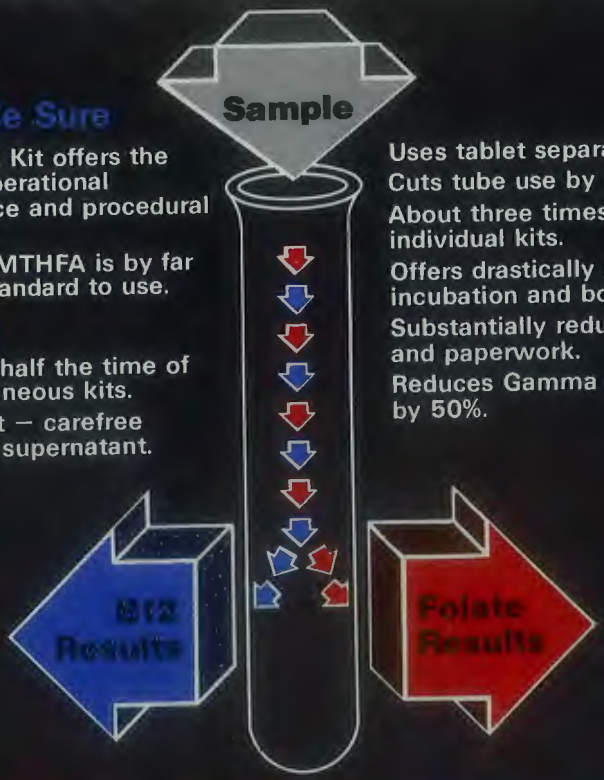

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The approach must also relate to the general examination system and perhaps a different hierachical system in the laboratory. In our situation where specialist qualification is usual these textbooks would provide little help in preparing for examinations.

Charge Technologists,
Diagnostic Laboratories,
Dunedin Hospital.

Mackie and McCartney Medical Microbiology, Volume I: Microbial Infections, 13th Edition, 1978, J. P. Duguid, B. P. Marmion, R. H. A. Swain 666 pages, illustrated. Published by Churchill Livingstone. Price $NZ32.50. Obtained from N. M. Peryer Ltd, Christchurch.

As explained in the preface the alteration of the title of this edition is intended to remind readers of the origins of this book. The use of the names of the original authors is intended to provide continuity as no doubt the editorial team will change from time to time. However, old habits die hard and I'm sure that it will be some time before one hears the standard text book referred to as "Mackie and McCartney" and not "Cruikshank."

The format of this edition is the same as its predecessor being divided into five parts.

Part one deals with the biology of microorganisms, sterilisation, and disinfection, antimicrobial agents and immunology. The content is the same as the previous edition although some new information has replaced old material and diagrams have slightly altered, and reduced in size (not to their detriment) so that the length remains the same as that of the 12th edition. This is true of all chapters and consequently the book contains the same number of pages as the last edition.

Part two is devoted to bacteria of medical importance. Each organism is described under the following headings: morphology, cultural characteristics, laboratory diagnosis, chemotherapy, epidemiology and control measures. Common bacterial pathogens are described in some detail, while less common organisms receive very little mention or are completely omitted. The sections on Donovania and *Streptobacillus moniliformis* have been completely omitted from this edition. There is no mention of *Campylobacter fetus* nor indeed of the causative organism of Legionnaires disease. Although there has been some reordering of material, some taxonomic changes and new references added basically there is very little difference in this edition.

Part three is concerned with the pathogenic viruses and associated diseases. Material here is very much the same as that contained in the 12th edition although a brief mention of the possible causal relationship between viruses and gastroenteritis has been included.

Part four includes chlamydia, rickettsia, mycoplasma, pathogenic fungi and protozoa.

Part five deals with the diagnosis, the treatment and control of infections. This section contains some very valuable practical information on the isolation of the causal organism and the antimicrobial treatment of infections.

However, the latter two parts remain the same as the previous edition.

This is a very useful textbook and will no doubt remain one of the standard texts for medical microbiology students.

"Medical Microbiology" Volumes I and II must be the most "used" books in diagnostic microbiology and indeed no laboratory should be without both copies. M.J.

Abstracts

Contributors: R. D. Allan, E. R. Crutch, L. M. Milligan

Clinical Biochemistry

Detection of Small Numbers of Monoclonal B Lymphocytes in the Blood of Patients with Lyphoma. Ault, K. A. (1979), *New. Eng. J. Med.* **300,** 1401.

More than 80 percent of lymphoid malignancies involve clonal proliferation of cells of the B lymphocyte class. The method is a cytofluorimetric detection of cells showing surface immunoglobulin of one light-chain class and can detect 10 percent or less of monoclonal B lymphocytes in normal blood. A rather sophisticated programme is used for the evaluation. The method is intended to check the result of treatment and to verify remission. R.D.A.

Preparation and Stability of a Liquid Creatinine Kinase Isoenzyme Control from Rabbit Serum. Lun, J. (1979), *Clin. Chem.* **25,** 873.

MB isozyme may be prepared *in vitro* from rabbit serum containing only MM and BB isozymes by means of a hybridization technique. The MM and BB dimers dissociate in 4mol/1 urea allowing random recombination of MB monomer. A liquid CK isozyme control can be made from mixtures of rabbit sera treated in this way and stabilised with glycerol and two mercaptoethanol. Stable for three months at 4°C.

—R.D.A.

CSF Enzymology, CK and LDH and Isozyme pattern as a Brain Damage Index. Viallard, J. L., Gauline, J., Dalens, B. and Dastugue, B. (1978), *Clin. Chim. Acta.* **89/3,** 405.

Blood and serum was examined from a young girl suffering from a convulsive episode of toxic origin. Elevated levels of both enzymes were found in the CSF. The CK was mainly in the BB form and the LDH in the H_4 form.

—R.D.A.

A Rapid and Inexpensive Laser Nephelometric Assay for Plasma Pregnancy Specific β, glyceroprotein Levels. Wood, P. J., Cockett, D. and Mason, P. (1978), *Clin. Chim. Acta.* **90/1,** 87.

Studies of this protein may prove to be of value in assessing foetal risk. A nephelometric method is suggested as a quick alternative to radial immunodiffusion or "rockets." Some plasmas produced high blank readings and this problem was overcome by a protamine sulphate precipitation step. —R.D.A.

CK Isozymes: Part 2. Technical Aspects. Baillie, E. E. (1979), *Laboratory Medicine,* **10,** 337.

This article discusses stability and compares methods. It details the preparation of a human control for CK isozymes using cubes of brain and heart vortexed in distilled water. It is also mentioned that nearly all postmortem sera from the left atrium or inferior vena cava contains all three fractions.

Agarose gel electrophoresis is favoured with visual interpretation of the resulting fluorescence. No CK-MB is detected in normal serum by this technique. No direct reference is made to the actual method but it could be deduced that it would be mentioned in an article by Morin, L. G. (1977), *Clin. Chem.* **21,** 1088. —R.D.A.

Phagocytic Myeloma Cells. Fitchen, J. H. and Stephen, L. (1979), *Am. J. clin. Path.* **71,** 722.

A case of a patient with otherwise typical multiple myeloma whose myeloma cells were phagocytic, is described. Phagocytosis of erythrocytes, erythrocyte precursers, lymphocytes and platelets was found. Ingestion of latex particles by myeloma cells was demonstrated *in vitro.* Authors' Abstract.

HLA-linked C_2 Deficiency in a Dutch Patient with Systemic Lupus Erythematosus. Berrens, L., Baart De La Faille, H. and Borst-eilers, E. (1979), *J. clin. Path.* **32,** 528.

A patient with SLE had undetectable levels of haemolytic complement, and an absence of functional C_2. The patient's relatives had half-normal values. Factor B levels were low in all cases and C_4 levels were raised in the relatives only. Tissue typing showed linkage of the C_2 deficient gene with the HLA-A10/B18 and A9/B18 haplotypes. The article was interesting also, in that references for the various techniques for assaying the complement components were given. —R.D.A.

Amylase to Creatinine Clearance Ratio in Renal Diseases. Andriulli, A., Bergia, R., Masaera, G., Baiardi, P., Pellagrina, S. and Tandala, M. (1979), *Gastroenterology,* **77,** 88.

Contradictory data on the specificity and diagnostic value of an elevated amylase to creatinine clearance ratio during acute pancreatitis, may be due to a lack of knowledge of clearance mechanisms. Experimental studies demonstrate tubular reabsorption contrary to previous reports. The authors confirm partial reabsorption of amylase in the tubules. In renal disease an increase of the ratio indicates a reduction of functioning nephrons or severe tubular damage. The elevation of the ratio in acute pancreatitis does not seem to be due to renal impairment. —R.D.A.

Haematology

Chromosome Analysis of Bone Marrow Samples from Country Patients. Bell, J. A. (1978), *Pathology,* **10,** 285.

This paper outlines a procedure for partially processing bone marrow samples from country areas before posting to the cytogenetic laboratory. The cytogenetic laboratory provides a "bone marrow kit."

A Micromethod Prothrombin Time for Oral Anticoagulant Control. Lam-Po-Tang. P. R. L. C., and Starr, Helen (1979), *Pathology,* **11,** 39.

A capillary prothrombin method is described

using the Australian Reference Thromboplastin. The method was used for the control of oral anticoagulant therapy in 59 patients on 225 occasions and was found to be reliable and convenient. The capillary prothrombin ratio could be correlated with the conventional plasma prothrombin ratio. —E.R.C.

An Improved Procedure for Accurate Assays of Factor VIII. Margolis, J. (1979), *Pathology* **11,** 149.

This paper details an improved one-stage method for accurate assays of factor VIII. Activated deficient plasma substrate (ADSP) is used as a single reagent for parallel tests. The coagulation time results are reproducible to 0.5 percent. —E.R.C.

Neutrophil Myeloperoxidase: A Simple, Reproducible Technique to Determine Activity. Kitahara, M., Simonian, Y. and Eyre, H. J. (1979), *J. Lab. and Clin. Med.* **93,** *232.*

Neutrophil myeloperoxidase deficiency has been rarely documented. This is because of the low incidence of infections in these individuals and the lack of use of various screening techniques which would detect them. Using the Hemalog D the authors have identified one completely deficient and 18 partially deficient subjects. E.R.C.

The Determination of Antithrombin III by Radioimmunoassay and its Clinical Application. Chan, Vivian, Chan, T. K., Wong, Vivian, Tso, S. C., and Todd, D. (1979), *Br. J. Haemat.* **41,** 563.

A radioimmunoassay has been developed for the determination of anti-thrombin III in man. Some clinical findings using this technique are described. —E.R.C.

Red Cell Enzyme Defects as Nondiseases and as Diseases. Beutler, E. (1979), *Blood* **54,** 1.

This review article discusses the majority of red cell enzymes with particular reference to whether or not a deficiency of certain enzymes actually produce haemolytic anaemias. —E.R.C.

A Comparative Study of Chromosome G—Banding Using Trysin, Papain and Pretreatment with Emulphogene. Khalid, G., Neumann, H., Flemans, R. J. and Hayhoe, F. G. J. (1979), *J. Clin. Path.* **32,** 482.

G-banding of chromosome metaphase preparations were performed with trysin, papain and pretreatment with the surface active agent, emulphogene. Using emulphogene as a preliminary to proteolytic digestion the authors found a general superiority of papain over trysin. —E.R.C.

Identification of Specific Antibodies to Extractable Nuclear Antigens by Passive Immunodiffusion. Kozin, F. and Fowler, Maralyn (1979), *Am. J. Clin. Pathol.* **71,** 437.

Extractable nuclear antigens have been identified in a number of systemic rheumatic diseases. The authors describe a simple immunodiffusion method to identify extractable nuclear antigens with high specificity and sensitivity that compares favourably with parallel haemagglatination studies. —E.R.C.

Techniques for Diagnosing Prethrombotic States. A Review, Sixma, J. J. (1978), *Thrombos. Haemostas. (Stuttgart)* **40,** 252.

The author reviews the tests currently available for detecting prethrombotic states. He concludes by stating "No reliable test is available for identifying a prethrombotic tendency in individual patients, however, these drawbacks do not mean that the tests are of no value." —E.R.C.

Immunohaematology

Auto-Immune Hemolytic Anaemia caused by Anti K-13. Marsh, W. L., Dinanoli, J. and Oyen, R. (1979), *Vox Sang* **36,** 174.

A case of auto-immune hemolytic anaemia caused by auto K-13 is described. Direct antiglobulin tests using monospecific reagents showed that IgG and the C3 component of complement were present on the patients red cells—Eluted auto-antibody did not react with Ko or K-13 cells. Kell Blood group antigenicity of the patient's red cells did not appear to be reduced. —L.M.M.

Coexistent TK and Va Polyagglutinability. Beck, M. L., Myers, M. M., Moulds, J., Pierce, S. R., Hardman, J., Wingham, J. and Bird, G. W. G. (1978), *Transfusion* **18,** 6.

Serologic investigations of the red blood cells of two patients indicated polyagglutination as the cause of the compatibility problems. Lectin studies to classify the variety of polyagglutination demonstrated the simultaneous exposure of two latent membrane receptors TK and VA. It is proposed that different bacterial enzymes were responsible. —L.M.M.

HLA Types and ABO Blood Groups in Patients with Infectious Mononucleosis. Rosdahl, N. and Svejgaard, A. (1979), *Tissue Antigens,* **13,** 223.

Investigations of HLA and Blood Groups were carried out on a number of patients with infectious mononucleosis comprising of all known cases diagnosed within one year in a restricted geographical area of Denmark. Some interesting statistical information is tabulated.

—L.M.M.

Distribution of H Antigens in Persons of Blood Groups A, B and AB. Lill, P. H., Stejskal, R. and Misna, J. (1979). *Vox Sang,* **36,** 159.

The distribution of H antigens in tissues of persons of blood groups A, B and AB was examined. H antigens could be demonstrated in all tissues in which the isologous antigens A and B were demonstrated. Since the results varied greatly from tissue to tissue, it was not possible to interpret the data with respect to the biochemical pathways of blood group antigen formation.

—L.M.M.

The Influence of Ionic Strength, Albumin and Incubation Time on The Sensitivity of the Indirect Coombs Test. *Jorgensen, J., Nielsen, M., Neilsen, C. B., Normark, J. (1979), Vox Sang,* **36,** 186.

The sensitivity of the indirect Coombs Test was investigated using saline, albumin or low ionic strength (LIS) in the incubation phase and a varied incubation time. Titration of a number of antibodies showed the LIS always resulted in a higher or almost the same mean scores as the other solutions after the same incubation time.

L.M.M.

Erratum

NZJ med. Lab. Technol. (1979), **33,** 41, line 20. Should read Factor VIII, IX, XI and XII.

Results, Case B should read, Patient PTT >240sec. Factor XII Assay <1 percent.

Line 38, should read Factor XII.

Page 55, line 4 should read mmol/l in each case.

May 1978 should read May 1979.

INDEX TO VOLUME 33

ABO Antibodies in Cryoprecipitate 75
ABO Blood Group Anomaly 5, 66
Abstracts 29, 61, 85, 93
Allan, R. D. 53
Anderson, R. A. N. 8
Austin, R. J. 76
Bingham, C. 48
Blood Group Survey of NZ Resident Cook Islanders 79
Book Reviews 26, 60, 97
Brooks, H. J. L. 20
Brown, T. J. 23
Chambers, T. L. 20
Chapman, C. 33
Chemotherapy of Primary Amoebic Meningo-encephalitis 23
Coplestone, J. C. 5
Correspondence 3, 59
Cryoglobulin; An Unusual Type 52
Cursons, R. T. M. 23
Daniels, J. 79
Davies, G. F. 52
Desmond, F. B. 36
Donald, J. J. 23
Erratum 66, 96
Factor XII Deficiencies; Two Unrelated Cases 40
Finlayson, J. 84
Folate Assay; L. casei 84
Fowler, R. T. 13
Gainsford, S. 42
Gill, M. B. 67
Hamer, J. 72
Hayden, B. J. 82
HJepatitis B: A New Haemagglutination Test 53
HDL Cholesterol in Serum 18
HCG in Urine; A Micromethod 53
Hurst, P. L. 54, 96
IgD Myeloma 7
IL Multistat III and Cholesterol Test Kit 54
Inolex Products for the Identification of Enterobacteriaceae 44
Instrument Calibration Survey in NZ Clinical Laboratories 36
Interlaboratory Surveys; Digoxin. A Case Study 13
Kappatos, D. 33
Kennett, K. 72
Key, E. A. 23
Knight, J. A. 35
Legge, M. 11
Lever, M. 13, 36
Low Ionic Strength Solution as a Red Cell Suspending Medium 76
McCarthy, M. D. 44
Mead, G. D. C. 90
Microsprum nanum Infection in the Arm of a Golfer 48
Milicich, G. C. 40
Millard, R. M. 18
Milligan, L. M. 75
Munster, D. J. 13, 36
Neisseria gonorrhoeae Comparison of Rapid Fermentation Test and Fluorescent Antibody Test 42
Newton, J. D. 8

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Mass concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre.
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CONTENTS

Address

T. H. PULLAR ADDRESS: BACK FROM BASICS TO THE TEA-LADY
M. B. Gill 67

Articles

A COMPARISON OF RED CELL ANTIBODY ELUTION METHODS
J. Hamer and K. Kennett 72

ABO ANTIBODIES IN CRYOPRECIPITATE
J. M. Well, J. A. Knight and L. M. Milligan 75

A REVIEW OF LOW IONIC STRENGTH SOLUTION (LISS) AS A RED CELL SUSPENDING MEDIUM
R. J. Austin 76

A BLOOD GROUP SURVEY OF NEW ZEALAND RESIDENT COOK ISLANDERS
J. Daniels and C. G. Storey 79

SUITABILITY OF COMMONLY USED ENRICHMENT BROTHS AND DILUENTS FOR ENUMERATING VIBRIO PARAHAEMOLYTICUS. A REVIEW OF CURRENT LITERATURE
B. J. Hayden 82

Technical Communications

AN UNUSUAL ISOLATE
J. Finlayson 84

L. CASEI FOLATE ASSAY
J. Tizzard 84

Abstracts

PAPERS READ AT A SCIENTIFIC MEETING OF THE CANTERBURY BRANCH OF THE NZACB AUGUST 1978 85

Reminiscence

IVOR W. SAUNDERS
G. D. C. Meads 90

Obituary 71

Book Reviews

Smith's Blood Diseases of Infancy and Childhood. Miller and Pearson 91
Cancer, Science and Society Cairns .. 92
Alba's Medical Technology Board Examination Review Vol 1. and II .. 92
Mackie and McCartney Medical Microbiology. Duguid, Mannion and Swain 92

Abstracts

Clinical Biochemistry 93
Haematology 94
Immunohaematology 95

Erratum 96

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