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T.H. Pullar Memorial Address 1977

Adaptation or Engineering?

D. J. Philip, FNZIMLT

When, some months ago, I received an invitation from the Institute Secretary to deliver this T. H. Pullar Memorial Address my first reaction was to feel tremendously honoured—for indeed an honour it is to be asked to pay the Institute's respect to their friend and mentor who played such a large part in our early history.

My ego being boosted by the invitation I quickly replied accepting. The second reaction followed almost as the reply letter slid from my hand into the mail box—PANIC. Thoughts of previous speakers with oratory prowess and masters of their subjects, my own puny abilities; What to speak on? These and other depressing thoughts flooded in. How does one choose a subject? Like Saul of old searching for the departed spirit of Samuel I felt almost a need to seek the advice and approval of the one in whose memory the address was to be given. In my own mind I did just that.

" Could I speak " I queried " on Education?" Assuredly—for Thos Pullar had devoted much of his energies towards **Education**—both for his pathology colleagues and the newly emerging technical group. Well I remember as a junior trainee in Auckland Hospital hearing Douglas Whillans, who was president of the Association of Bacteriologists at the time, speak in glowing terms of the pathologist from Palmerston North who was giving so much help in the preparation of new syllabi—particularly at that time the about-to-be-introduced Intermediate syllabus. These were not days when the meetings for discussing these new syllabi were held in working hours—people's own weekends were given up and Thos Pullar freely gave of his own time.

Could I speak my mind I enquired of the mythical departed spirit on **Examinations?** Once again vibrations gave me a strong affirmative answer. Thos Pullar was for many years an examiner for our examinations. Well I recall those brown eyes looking at me over the examination bench as he coaxed from me facts known by me but poorly remembered in the heat of an examination. No roaring, no ranting, no sarcasm (enough of this came from another famous personality) but the gentle investigation of a skilled examiner well versed in not only the theory of this work but the practical aspects as well.

Laboratory Management—Would this be a subject approved by the departed spirit? Again a positive reply. It was never my privilege to work with Thos Pullar but I have spoken with those who did and have heard only high praise. Recently I had occasion to consult some Conditions of Employment drawn up by Dr Pullar and they showed the depth of understanding of this man. Conditions not so detailed as to be restrictive—not so broad as to be useless but at a level which allowed for all contigencies.

This skill in management in no way overshadowed Dr Pullar's **academic skills.** In fact of course they complemented each other and Dr Pullar won his colleagues praise and commendation for his work as a practising pathologist. Clearly the spirit would allow me to be academic even though my own confidence was much more reserved in its allowances.

Could I just speak on a warm humorous level? Ah yes the Spirit would allow this for it spoke for a man who above all else was a warm caring humane person. After Dr Pullar shifted to Tauranga I had the privilege of visiting him for a morning in his laboratory. It is a memory I cherish. As he showed me around it was obvious he had a real understanding of technical matters. Instruments were not foreign to him—we could discuss them in their technical aspects—he was knowledgeable. We sat together over morning tea and something of the warmth, the humanity and the quiet sense of humour came through.

He was indeed a man of many parts and as in my mind I consulted with him these many facets did not really help me because I had warrant to speak on virtually any aspect of laboratory work. Dare I try to blend them all together? Probably I shouldn't but this is what I am going to attempt.

Several years ago Lynn Margulis in an article on symbiosis and evolution reviewed some of

the then current thoughts and possibilities on evolution as related to apparently dependent symbiotic partners.

If we accept that every form of life be it birch, bee or bacterium shares a common ancestry then we are left with finding an answer to the profound biological schism that exists—namely that all living things belong to one or other of two groups that are mutually exclusive. Either they are organisms with cells that have nuclei, or organisms with cells that do not (I except of course viruses and virus-like particles which can only proliferate within living cells).

Every first year trainee is familiar within bacteriology with the division of the procaryotic and eucaryotic organisms. Everyone is aware of whether cells contain their genetic material organised into chromosomes within a membrane enclosed nucleus, or whether the genetic material is dispersed throughout the cytoplasm. We are all aware of the fundamental relatedness of living things with their almost identical nucleic acids, enzyme systems, and proteins made up of the same amino acid units. Yet we still have this profound division.

Excluding parasitic relationships we see mutually advantageous relationships between two or more organisms—a relationship we know as symbiosis. A good example is the leguminous plants with their root nodule. With the plant roots developing infection threads certain free living soil bacteria are transported into the cytoplasm of the root cells where as bacterioids they combine with the host cell to form a specialised tissue capable of fixing inert atmospheric nitrogen—something neither organism could do on its own. This endosymbiotic relationship is not hereditary and each generation of legumes must establish its own relationship with a new generation of bacteria. However, hereditary endosymbiosis does occur in nature with quite surprising regularity. A plant such as *Psychotria bacteriophila* containing the bacterial symbiont within its seed is able to pass on to its progeny not only its own chromosomes and cytoplasm but the bacteria's as well. The lichens demonstrate such a symbiotic partnership with an autotrophic alga and a heterotrophic fungus. Work has been done to dissociate the partners in one group of lichens and raise the component independently. A well-known paramecium is characteristically green because of numerous photosynthetic green algae which inhabit its cell. These algae are able to survive indefinitely if removed from their host and are also able under near starvation conditions to provide sufficient in nutrients to their host by their own photosynthesis. Interestingly when the two symbionts are reconstituted after separation the algae multiply only up to what appears to be a genetically regulated number. Should free living algae of the same genus be encountered by the paramecium they are promptly ingested and digested—something to which its own algae partners are not subjected. Even with E.M. differences are not apparent to the observer.

These are examples of a simple symbiosis involving hosts and their symbionts who all belong to a single species. However, symbiotic relationships of far greater complexity are known. The flagellate Myxotricha which inhabits the gut of certain Australian termites is an example of a complex symbiotic relationship. This protozoon, itself a symbiont, is at the same time a host to three other symbionts. Originally thought to be a multi-flagellated organism it has now been shown to have only a few normal flagella at one end and the rest of what was thought to be flagella are in fact spirochaetes which in turn are symbiotically associated with further smaller spirochaetes whilst inside the cell other bacteria live in perfect harmony with their host. We have then, in brief, seen how different independent organisms can enter symbiotic partnerships which are sometimes perpetuated on a hereditary basis.

What is the situation in the eucaryotic cell. Take for instance the cell of a normal green leaf. Not only does it have a nucleus but it has other organelles—its **chloroplast,** master of the photosynthesis chemistry, and its **mitochondria** producing ATP (adenosine triphosphate) to trigger and power biochemical reactions, to mention but two. The intriguing question that springs immediately to mind is to know if these organelles could have originally been independent organisms. There is some evidence to suggest that this was so. The presence of cytoplasmic genes notably in association with chloroplasts and mitochondria gives

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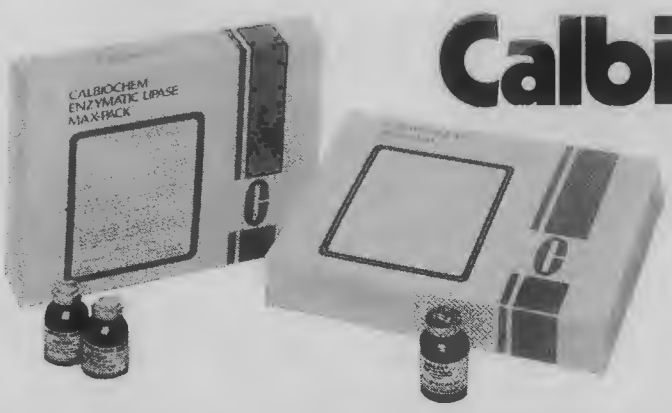

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some real evidence to the possibility of earlier independent organisms now combined in a hereditary endosymbiosis.

Traditionally of course we associate genes with the DNA in the chromosomes of the cell nucleus. However plastids, to which chloroplasts belong have a DNA of their own unrelated to the DNA of the cell nucleus. Not only is this DNA present but chloroplasts have their own messenger RNA, transfer RNA, ribosomes and other factors necessary for independent protein synthesis. Similarly mitochondria have their own protein synthesizing mechanism which however can produce only a fraction of the required proteins and enzymes required by the organelle in order to function.

Why within a eucaryotic cell with its own nuclear DNA directing almost all the protein synthesis should there be separate protein synthesis within the organelles? Does this ability, combined with the ability to grow and divide within the cell imply that these organelles were once free living organisms that are now firmly established in hereditary endosymbiotic relationship? This theory is not new of course and was advanced in the 1920s only to fall into disrepute because of doubtful data used to defend the then novel concept. Today there seems to be more data for its defence. Some biologists would look at a third group of organelles, the flagella and cilia and argue that these became associated with the eucaryotic cell in much the same way and the fibril pattern of flagella and cilia would support this. One cannot help but notice that the ribosome size of the procaryotes is 70S and that the ribosome size of the eucaryote is 80S **except** for the ribosomes of its mitochondria and chloroplasts which are the same 70S as the procaryotic cell. The question of the symbiosis theory as an explanation of the evolution of the lower organisms is not the purpose of this address nor indeed is there yet any conclusive proof that it provides anything other than a speculative evolutionary scheme. What I really want us to notice at this point is the ability of organisms both on a hereditary and non-hereditary basis to live in endosymbiosis to the mutual benefit of both, or rather all, of the symbionts. Let us move our attention slightly to look at the evolution of R factors in bacteria. We need to briefly define two entities or genetic elements. An episome is by definition an independent genetic element which occurs in addition to the normal cell genome which can be transmitted to other cells, and which can replicate **either** as an autonomous unit or as one integrated into the host genome—an example is the F factor. Another group of genetic elements called plasmids never become integrated into the host chromosome but remain as independent self replicating units. Plasmids are known that are responsible for the synthesis of certain bacteriocines and of the so called resistance transfer factors (RTF) which confer resistance to antibiotics. Plasmids may also be transferred by cell-to-cell contact. In an article in Bacteriological Reviews of September last year Darryl Reanney of our own Lincoln College examines "Extrachromosomal Elements as Possible Agents of Adaption and Development" defining these elements in an all embracing catholic term to mean all extrachromosomal genetic elements which includes of course the transfer factors.

Discussing streptomycin resistance the article states "During recent decades, man has created a stress situation for certain bacteria by introducing into specific ecosystems very high concentrations of antibiotics. All theories of evolution predict that bacteria should adapt to this stress by the acquisition of drug resistance. It is central to the thesis of this article to note that the type of resistance to a drug such as streptomycin developed in the laboratory differs fundamentally from that acquired in nature. Under laboratory conditions, high concentrations of streptomycin select from modulated mutants of *E. coli* in which the 30S ribosomal subunit has been modified. By contrast, the drug-resistant bacteria isolated from environments such as hospitals contain plasmid (R factor) DNA carrying genes for streptomycinases: such R factors inactivate streptomycin.

"Further, and here it is the key point, extrachromosomal elements (ECE's) have the crucial ability to replicate independently of (and in relaxed strains many times faster than) the genome of the cell. Hence, once a plasmid carrying a gene enters a selectively disadvantaged population, its spread can assume an

epidemic character. In this context it is easy to see how selection has created the infective ability of many ECE's, not as a means of bringing about cell death, but as an extraordinarily efficient mechanism of accelerated cell evolution."

Later in the article speaking about the evolution of R factors the writer states "A characteristic genetic feature of most R factors is the presence of genes coding for proteins that inactivate antibiotics. The origin of such genes has not been unequivocally determined, but Benveniste and Davies have shown that the antibiotic-modifying enzyme of certain Actinomycetes species are similar to those responsible for plasmid-mediated drug resistance. The presence of such enzymes might be of permanent selective advantage to a soil organism. No known plasmid can infect both enteric bacteria and Actinomycetes, but the transfer of genes between such taxonomically distant genera may have been accomplished by using intervening species in a 'stepping stone' manner. If soil Actinomycetes do constitute the ancestral reservoir for resistance genes, then the contemporary presence of these genes in so many diverse species inhabiting different eco-systems points to a widspread process of gene dissemination in nature.

"In the context it is useful to examine the specific example of the penicillinase—TEM β lactamase—in the light of recent data. Hedges *et al.* found that the TEM protein was similar in a wide variety of naturally occurring plasmids, irrespective of the species of origin or the geographical source of the R factor. These data strongly suggest that (i) the TEM gene for all these R factors had a common origin and hence (ii) this gene must be able to migrate among heterologous DNA's.

"Resistance genes with this ability have now been discovered. Hedges and Jacob showed that the ampicillin resistance gene on plasmid RP4 could be acquired by other plasmids resident in *E. coli.*"

I am sure by this time many must be asking themselves when this boring old fool is going to stop repeating things with which all microbiologists are extremely familiar and that others can find in standard texts and journal articles.

The point that hopefully I have been able to arrive at is that organisms in an adaptive manner are able to symbiotically acquire to themselves factors which enable them to protect themselves against life — threatening outside influences. And the intriguing premise to me is that these factors are not necessarily the result of some genetic mutation but mark the acquisition of a resistance gene which has been available for an incalculable time in another soil organism.

I want to move from this subject to what our Editor some years ago whimsically referred to as "Philip's Physiological Phables" but before I do so I want to pause ever so briefly to remind ourselves of another way modern science has given to us to alter genetic structures of organisms—I refer to GENETIC ENGINEERING. The year 1976 marked the real beginning of the era of genetic engineering. Khorana from the Massachusetts Institute of Technology announced that he had successfully synthesized the first fully functional gene from off-the-shelf chemicals and allowed as Clifford Brobstein said a new "genie" to emerge from the bottle of scientific research. The ability to split open the DNA in an organism and insert new genes together with the synthesis of man-made genes has opened doors of genetical engineering that has occasioned both here and overseas not only criticism but concern. That there is much benefit that might derive from this new science is not denied but the inherent dangers gives reason for pause and a long look. I want to say no more about genetic manipulation in this way save to observe that it seems to me that here, rather than the organism adapting itself to meet requirements for its own survival, we have it dancing as it were as a puppet on a string to meet the so-called needs of a master completely outside of itself. This is no happy symbiotic arrangement to the undoubted advantage of the organism but the autocratic imposition of hereditary factors which may or may not be to the organisms advantage and which in fact may work not only to its disadvantage but to the disadvantage of others, even its creators.

For a few minutes now to conclude this address I would like to turn from the adaptive processes organisms have used to ensure their survival to look at our need as a body of

medical technologists to adapt to ensure our survival.

I personally do not find the evolutionary theory satisfying and look to a record of special creation to fill the many gaps that evolution cannot explain. In doing this, however, I certainly hold to the need, and the ability, of organisms to adapt to their environment whilst still remaining within that overall premise of the Genesis record that says that each created unit of life will reproduce "**after its kind.**"

Homiletically, I transfer this concept over to our need to continue to produce medical technologists "after our own kind"—technologists who will be able to hold the pride of place that New Zealand technologists presently hold with the world of medical laboratory technology and if that sounds conceited I can assure you that those of us who sit on the Technologist's Board and have occasion to examine overseas qualifications for recognition here in New Zealand are constantly reminded of the fact that New Zealand trained technologists need have no inferiority complex about their position within the world scene of Medical Laboratory Technology.

However, I would be quick to emphasise that this is a changing scene and that as needs charge and as external requirements are altered we will need to be ever vigilant to adapt to these external circumstances to produce "after our kind" and maintain our position.

The point that I would like to make today and make as strongly as I can is that this adaptation must be completely under our own control. We must, as it were, introduce only the new genetic material that we require to maintain and improve our own body—this organism known as a Medical Technologist. We must not allow changes to be dictated by some outside "genetic engineer" who may work to our advantage but who may be far more interested in promoting his own ends. And lest there be some who think that these are the words of an alarmist out of touch with reality let me refer you to the scene in the United Kingdom at the moment and in doing so I am indebted to a New Zealand technologist who over the last year has attended a course in Laboratory Management at the Paddington College School of Medical Laboratory Sciences.

Walter Finch, head of this school and deputy principal of the College, himself a Medical Technologist, expressed grave concern to his students about the trend that training and examinations were taking in the United Kingdom. However, his greater concern was the attitude of the vast majority of the Institute members. Apart from the older group whose names we have come to know over the years (men such as Baker) there is a widespread general, if not apathy, then, disinterest. He issued his students with a list of bodies interested in the education and training of Medical Laboratory Technicians in England and Wales—22 in all and as diverse as the Institute of Medical Laboratory Sciences and the Royal College of Pathologists through regional and health authorities, joint committees, teacher organisations and polytechnics, and various councils down to trade unions and commercial and industrial interests. Many of these groups would surely come within my definition of genetical engineers. As I understand it the Technician Education Council proposes to introduce this year a course which will be lower than the present HNC—a course that we have already found to be lower than the previous very high standards of the Institute in the United Kingdom.

It is not my purpose to argue the merits or demerits of their course. The essential factor I want us to note is that whilst in early stages Institute members there had some say in the formulation of courses their final representation at the TEC level is a scientist drawn from the timber industry and a food technologist! This must not be allowed to happen here in New Zealand. We must gain and maintain completely the control of our own destiny. In our egalitarian society we could easily lose the control. Already on the political side I have been saddened to see that we have had conditions imposed on us for which we did not ask and which we may not want. Standard conditions negotiated outside of our Institute are suddenly thrust on us with no reference to our representatives at all. Not all these conditions are advantageous to us as witness the travelling allowance and indeed the minimum nine hour break.

Currently there is work going on towards the formulation of a new diploma course and

we shall no doubt be hearing more of that later today. One of the main reasons for proposing this course was the dissatisfaction we had with the structure of courses offered to us through the TCA. Our earliest representations to them, which I well remember, produced a course which was not exactly what we wanted but which fitted in with other courses that TCA were offering. With hindsight we can see that we should have tenaciously held out for exactly what we wanted. I believe we are today in a much stronger position to ensure that we adapt in our own way and in such a way that we get exactly what we want. But we will need to be watchful. I applaud the decision of the Society of Pathologists not to oppose the restructuring of the Medical Technologists Board to allow Medical Technologists to have an absolute majority. This is right and proper and their decision recognises the growing maturity of the Institute and not only its right, but its ability, to be able to be in control of their own affairs. As the current Chairman of the Board I would, however, like to add quickly that I hope the Society will continue to give us the invaluable aid and advice that their members on the Board afford. But outside of this Board there are some who would not hesitate to use any proposed changes in training and examination to their own ends. It is not the purpose of this address to name the genetical engineers but to warn that they are there. How can we best ensure that we are masters of our own adaptation. I believe the answer in the first instance is quite clear. We must support to the very best of our ability our own Institute. And if this whole address now sounds like a giant plug for the Institute I do it quite unashamedly (and I hasten to add with no prompting from the Council). I believe we have in those members we will elect today men (and a woman) who will serve us well. But their effectiveness both through the Institute and the Technologist's Board will be governed by the support that you and I give it—by the interest, constructive criticism and enthusiasm we display. If we give this sort of support; if we carefully choose as it were the extrachromosomal elements that will enable us to successfully adapt and if we resist the outside genetical engineers who would perchance adapt us to their own ends then I believe that we will be doing those sorts of things that would have very much pleased the one in whose honour this address is presented—THOMAS HENRY PULLAR.

A Case of Tuberculous Synovitis

Helen Brady

Microbiology Division, Department of Laboratory Services, Wellington Hospital

Received for publication, June, 1977

Introduction

The greater proportion of diseases caused by the mycobacteria in man in New Zealand is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). However, other members of the genus Mycobacteria may infect humans; the commonest source for these being animals[2] Ekdahl (1970). *Mycobacterium bovis* was originally isolated from tubercles in cattle and in New Zealand it has also been found in opossums, deer and feral pigs. *Mycobacterium marinum* may be present in tropical fish and the surrounding water in which they live and *Mycobacterium scrofulaceum* has been found to be present in swimming pool water, subsequently causing cervical adenitis in children.

Case History

In April 1976 a Caucasian male aged 27 years had a splinter removed from the base of his left ring finger. Three months later after receiving a knock at work, the finger showed signs of swelling and steroid injections were given. After another three months the finger again became swollen and painful with movement being reduced. The patient was given tetracycline, ampicillin and cloxacillin. Mr A. A. R. Griffin, Orthopaedic Surgeon of Wellington Hospital, performed an exploratory

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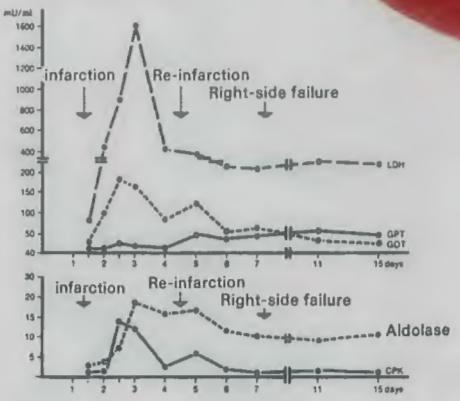

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operation. During the operation, tissue and swabs from the tendon sheath were taken and later sent to the laboratory.

Sections of this tissue showed a solitary acid alcohol fast (AAFB) bacillus and a diagnosis of tuberculous synovitis was made. The patient was started on anti-tuberculous therapy; 1g streptomycin intramuscularly daily and PAS INAH-D-1 envelope twice daily. On discharge from hospital four weeks after admission, the treatment was 1g streptomycin intramuscularly twice weekly and PAS INAH-D-1 envelope twice daily. After a reaction to streptomycin the treatment was again changed to rifampicin 450mg daily and PAS INAH continued as stated. The finger became fully functional and the patient, nine months after initial injury, resumed work.

Isolation

The addition of 0.2% pyruvic acid to Lowenstein Jensen (LJ) medium is recommended for the isolation of *M. bovis*. Bott (1970)[1]. A specimen of the tendon tissue was received in the tubercle laboratory and ground completely using a Sorvall Mixer. The liquid specimen was then centrifuged at 3000 rpm for 15 minutes and cultured onto the following media.

Media	Incubation Temp.
LJ plus glycerol	37°C
LJ plus 0.2% pyruvic acid	22°C, 30°C, 37°C
Middlebrooks 7H11 Agar	5% CO_2 at 37°C

The swabs received were also cultured and incubated as above. After six weeks small smooth pale cream colonies were seen on LJ containing 0.2% pyruvic acid incubated at 37°C. The other slopes showed no growth after 12 weeks incubation and were discarded as negative. Ordinary routine culture showed no growth.

Identification

Kinyoun's Modified Ziehl Neelson	—Small acid alcohol fast bacilli containing one metachromatic granule
Cording	—Not seen
Niacin Production	—Negative
Nitrate Reduction	—Negative
Pigment Production	—Non photochromogenic
Growth on Thiophen-2-carboxylic acid anhyrazide 10μm/m (TCH)	—Sensitive
Tween 80 Hydrolysis	—Negative
Semi quantitative Catalase	—Very slow 45mm
Arylsulfatase—3 days	—Negative
—2 weeks	—Negative

Animal Inoculation

When performing this test it is essential to standardise the inoculum and that the inoculum is diluted enough to produce a progressive infection in the animal being used. The culture was grown on a LJ plus 0.2% pyruvic acid slope for 4 weeks producing a good actively growing culture. In a safety cabinet, the organisms were "rubbed off" the slope into the water of condensation. To produce as smooth a suspension of organisms as possible, the fluid was transferred into a Universal container with a puncture cap and broken up by carefully passing it through a tuberculin syringe several times. The fluid was made up to 10ml with Ringers solution and left for an hour to allow the larger clumps of organisms still present to settle. The supernatant was removed and a 1/10 then a 1/100 dilution was made again in Ringers solutions. The latter dilution should be equivalent to approximately 0.001mg of wet weight of the culture. As the above biochemical reactions suggested that the organism may be *Mycobacterium bovis* a rabbit was the animal of choice. 1ml of 1/100 dilution was injected intravenously using the large ear vein of the rabbit. After 4 weeks the rabbit died and a post mortem was performed. A generalised infection involving lungs, kidneys, liver and spleen had been produced. Acid alcohol fast bacilli were isolated from all these organs and the above tests were performed on the cultures.

An identification of *Mycobacterium bovis* was made. This identification was confirmed by the Reference Centre for Atypical Mycobacteria at Wallaceville Animal Research Station.

Discussion

The important features of this identification were:

(a) The primary isolation of dysgonic colonies on LJ containing 0.2% pyruvic acid.
(b) Absence of cording.
(c) Lack of Niacin and Nitrate Reduction.

TABLE 1

SPECIES	GROWTH IN LESS 7 DAYS	PIGMENT PRODUCTION	NIACIN PRODUCTION	NITRATE REDUCTION	CATALASE RT	CATALASE MM	TWEEN 80 HYDROLYSIS DAYS	ARYLSULFATASE 3D	ARYLSULFATASE 2WK	ARYLSULFATASE TCH
M. tuberculosis	-	-	+	+	S	<40	5	-	-	R
M. bovis	-	-	-	-	S	<40	5	-	-	S
M. marinum	±	+	-	-	S	<40	5	-	+	R
M. scrofulaceum	-	+	-	-	R	>40	-	-	-	R
M. gordonae	-	+	-	-	R	>40	5-10	-	+	R
M. szulgai	-	+	-	+	R	>40	5	-	+	R
M. ulcerans	-	-	-	-	R	>40	-	-	-	-
M. chelonei	+	-	-	-	R	>40	-	+	+	R

Key S - Slow or sensitive

R - Rapid or resistant

Table 1 shows how the other mycobacteria which might have been implicated were excluded. Vestal (1975)[3].

Reported cases of *M. bovis* have usually been associated with the patients' occupations, abattoir workers, veterinarians, opossum hunters, farmers or leisure time activities, such as deer or opossum hunting. Butchers used to develop lesions called "butchers warts" cause by *M. bovis*. In this particular case, the patient, a builder, had killed and skinned opossums on his property and as these animals are known to carry *M. bovis* it is presumed that the organism was acquired from this source. Also in this laboratory we have had a case of a person keeping tropical fish becoming infected with *Mycobacterium marinum*. This case was interesting in itself as the Department of Agriculture and Fisheries had found that some of the owner's fish had "Fish TB." The tank water, which was very rarely completely changed, provided a good habitat for *M. marinum*.

Conclusion

Although New Zealand's temperate climate does not favour skin infections caused by the atypical mycobacteria such as *M. ulcerans* or *M. marinum* their possible presence must not be forgotten. Also the likelihood of members of the Genus Mycobacteria being involved in infections other than of pulmonary origin must be remembered. The best precaution to take when working with the mycobacteria is to put up culture media at varying temperatures on all specimens such as lymph nodes, pus, lesion material and tissues. It is also recommended that these specimens should not if possible undergo any decontamination process as it is a reported fact that this procedure can kill a certain proportion of mycobacteria present in the specimen.

I would like to thank Mr A. A. R. Griffin, Orthopaedic Surgeon of Wellington Hospital, for permission to publish this case history and Doctor J. D. Manning, Medical Microbiologist, Wellington Hospital, for his advice in the identification of this organism.

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Diagnosis and Identification of the Aetiological Agents of Primary Amoebic Meningo-encephalitis (PAM)

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From a paper read to the Annual NZIMLT Conference, Queenstown, 1977

Summary

The history of interest in amoebic meningo-encephalitis is outlined. The diagnosis and identification of *Naegleria* and *Acanthamoeba* infections is described and the characteristics of the diseases caused and aetiological agents tabulated. It is concluded that definable species of *Acanthamoeba* and *Naegleria* are established as pathogens of man.

Introduction

Traditionally the only amoeba recognised as being pathogenic to man has been the aetiological agent of amoebic dystentery *Entamoeba histolytica.* This preoccupation with amoebic intestinal disease effectively inhibited consideration of the role of free-living amoebae as disease agents in other parts of the body until the relatively recent accidental discovery of cytopathogenicity in cell cultures by free-living amoebae some 20 years ago (Jahnes *et al,* 1957[7]; Culbertson *et al,* 1958[4]). Subsequently they have been shown to be responsible for a multitude of diseases in both man and animals, ranging from chronic illnesses such as respiratory infections and blindness, to the very acute disease of Primary Amoebic Meningo-encephalitis (PAM) (Chang, 1974[1]; Visvesvara *et al,* 1975[9]). Taxonomically, they belong to the genus Naegleria within the family *Schizopyrenidae,* and to the genus Acanthamoeba within the family *Acanthamoebidae.* They are ubiquitous in soil and freshwater environments throughout the world where they normally exist as saprophytic organisms feeding mainly on bacteria, yeasts and organic debris. From an epidemiological viewpoint, the diseases they cause can be divided into two main groups: a swimming-associated acute meningo-encephalitis (caused by *Naegleria fowleri*) and non-swimming-associated chronic infections (caused by pathogenic *Acanthamoeba spp.*).

Materials and Methods

Pages Amoeba Saline (Page, 1967[8]).

$NaCl = 0.12g\ l^{-1}$
$MgSO_4.7H_2O = 0.004g\ l^{-1}$
$CaCl_2.2H_2O = 0.004g\ l^{-1}$
pH = 6.8
$Na_2\ HPO_4 = 0.142g\ l^{-1}$
$KH_2PO_4 = 0.136g\ l^{-1}$
Agar = 15g l^{-1}

CYM Medium (modified)

Glucose = 10.0g
Difco Yeast Extract = 5.0g
Difco Casitone = 10.0g
L-Methionine = 0.08g
d-Biotin = 0.002g
Thiamine HCl = 0.001g
Vit B12 = 0.000001g
Pages Amoeba Saline = $1000cm^3$
pH 6.8; Autoclave 15lbs/sq in/15min.
To $4.5cm^3$ of CYM, add aseptically $0.5cm^3$ of the following cocktail:
Sterile serum = $50.0cm^3$
Sterile hemin (01%W/V) = $10.0cm^3$
Sterile water = $40.cm^3$
Penicillin/Streptomycin. 200,000 units cm^{-3}

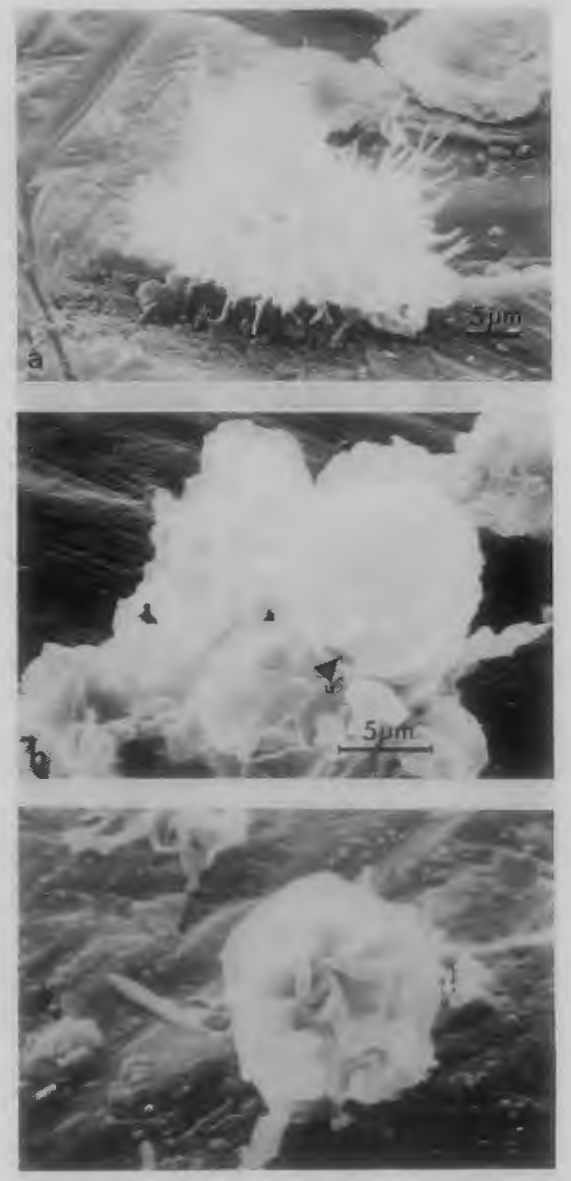
Results and Discussion

Diagnosis and Identification of Naegleria Infections. — *Naegleria* infections are usually characterised by some previous history of swimming in freshwater, and in New Zealand particularly, thermal pools, some 7-14 days before expressing acute typical meningitis symptoms. The aetiological agent is *Naegleria fowleri* which exhibits a tri-life cycle of trophozoite, flagellate and cyst stage (Fig. 1). Both the trophozoite and flagellate stages have been shown to be infective (Culbertson, 1971[3]).

The clinical isolation of amoebae from suspected cases may be achieved by cultivation of spinal fluid, brain tissue, or nasal discharge on Page's Amoeba Saline agar spread previously

Fig. 1. — Scanning electronmicrographs of *Naegleria fowleri*: (a) trophozoite; (b) cyst, arrow indicates the ostiole; and (c) flagellate stage.

Fig. 2. — Scanning electronmicrographs of *Acanthamoeba*: (a) trophozoite of *A. castellanii*; (b) cyst of *A. culbertsoni*, arrow indicates ostiole; and (c) cyst of *A. castellanii*.



with a lawn of live *E. coli* or *E. cloacae*, by axenic CYM culture, or by passaging suspected material through cell cultures, and incubating at 37-45°C. Samples may be centrifuged at 1500 rpm (500g) at room temperature, but it should be emphasised that at no stage should suspected samples be stored in the cold. The finding of amoebae (15-30μm) with a clearly defined ectoplasm and endoplasm, possessing a distinctive nucleolus, exhibiting eruptive broad pseudopodial movement, possessing smooth round cysts and a temporary flagellate stage is sufficient to make a tentative identification of *Naegleria* (Table I). Flagellation may be

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Table I. A comparison of pathogenic Naegleria and Acanthamoeba and the diseases caused by them.

	Naegleria	Acanthamoeba
Clinical Disease	Acute Primary Amoebic Meningo-encephalitis	Normally Chronic, prolonged nonspecific diseases
Symptoms	Severe headache, Nausea, Vomiting, Fever (39-41°C), Stiff Neck. Coma—Death	Nonspecific, Chronic Debilitating Disease
Epidemiology	History of Good Health—Recent history of swimming in freshwater, especially thermal pools in N.Z.	History of poor health. No history of swimming
Portal of Entry	Olfactory Neuroepithelium	Oral route and olfactory neuro-epithelium
Incubation Period	4-10 days	Uncertain; > 10 days
Onset	Fast	Slow and insidious
Organs Affected	Brain	Systemic
Pathology	Haemorrhagic necrotiszing meningo-encephalitis. Brain oedema. Perivascular collection of amoebae.	Chronic granulomatous encephalitis with focal necrosis
Amoebic Forms	Only trophozoites	Trophozoites + cysts
Clinical Isolation	Nasal discharge, CSF, and Brain	Nasal discharge and affected organs
Aetiological Agent	*Naegleria fowleri*	*Acanthamoeba culbertsoni* *Acanthamoeba castellanii* *Acanthamoeba polyphaga*
Protozoology	Trophozoites: 15-30μm. distinctive nucleolus, broad pseudopods	Trophozoites: 30-45μm distinctive nucleolus and water expulsion vesicles; very fine acanthapodia
	movement: eruptive and alternating	movement: very slow
	flagellate at 43°C: Yes cyst—spherical	flagellate: no cyst—wrinkled stellate, double wall
Growth Characteristics	Growth at 45°C on AS agar	Growth at 43°C on AS agar + 1% salt (*A. culbertsoni*)
		Growth at 37°C on AS agar (*A. castellanii, A. polyphaga*)
	Forms cytopathic effects in Vero cell culture	Forms cytopathic effects in Vero cell culture

achieved by flooding plates containing trophozoites, with sterile distilled water and incubating at 37-43°C for 90 minutes, whilst encystment is achieved by allowing plates to dry out. The use of a phase contrast microscope (about 200-300 Xs) is strongly recommended.

Diagnosis and Identification of Acanthamoeba Infections. — *Acanthamoeba* infections are usually nonspecific and chronic. There appears to be a need for some predisposing condition such as immuno-suppressive therapy and thus it is thought that pathogenic *Acanthamoeba spp.* are opportunistic pathogens. Recent unpublished work by the authors has shown that unheated normal human sera is capable of neutralising pathogenic Acanthamoebae at a titre of 1/20-1/40. This titre agrees well with

the indirect fluorescent antibody titres in over 180 normal human serum samples tested.

With the exception of the pathogenic species *A. culbertsoni* which produces an acute meningo-encephalitis, the other known pathogenic species (*A. castellanii, A. polyphaga*) produce chronic diseases in line with their lower virulence. The clinical isolation of suspected material is the same as with *Naegleria* infections. Microscopically, *Acanthamoeba spp.* can be differentiated from *Naegleria spp.* in that there is no temporary flagellate stage, the trophozoites (30-45μm) have no such clearly defined ectoplasm and endoplasm, nor do they exhibit eruptive movement but possess slender acanthapodia, and their cysts consist usually of a wrinkled ectocyst enclosing a stellate endocyst (Fig. 2). Table I is a supplement to the identification strategy used by the authors in differentiating between pathogenic and non-pathogenic *Naegleria* and *Acanthamoeba* from New Zealand thermal pools (Cursons *et al*, 1976[5]).

Conclusion

There is now sufficient evidence to include certain species of Acanthamoeba and Naegleria as pathogenic protozoan species of man. The number of reported isolations combined with an increasing number of retrospective diagnoses suggests that the apparently low incidence of reported infections may in fact be due to poor diagnosis in that free-living amoebae are never suspected. The recent finding of pathogenic *Acanthamoeba spp.* causing progressive corneal ulcerations (Visvesvara *et al*, 1975[9]) and the isolation of the so-called Ryan virus (Eldrige *et al*, 1967[6]) and Lipovirus (Chang *et al*, 1966[2]) in the mid 1960s (both of which turned out to be *A. castellanii*) from respiratory infections serve to highlight the variety of diseases that these organisms can cause.

Acknowledgments

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Evaluation of a Serum Latex Pregnancy Test

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Summary

Pregnancy testing in hospital is often more complicated than simply confirmation of pregnancy. The progress of hydatidiform mole and choriocarcinoma are monitored by quantitative HCG levels.

Threatened or incomplete abortions require a rapid test procedure prior to D and C. Clean urine specimens free from blood contamination, are often difficult to obtain from many of these cases.

A fast reliable HCG test that could be used on either serum or urine would enable the problem of blood contaminated urine to be overcome.

The new DAP test Macro, is able to be used on either serum or urine and an investigation was instigated to see if the advertised criteria were upheld.

Introduction

Immunological tests for the diagnosis of pregnancy depend on the detection of increased quantities of chorionic gonadotrophin present in the urine or serum of the pregnant woman. The excretion of human chorionic gonadotrophin (HCG) [1, 2, 7] is significantly raised

within one week after the first missed period and reliable positive results in immunological tests may be expected within four weeks of conception.

Early detection of pregnancy is important when a patient is to be subjected to therapeutic or diagnostic radiology or when ectopic pregnancy is considered. In these cases, a thorough understanding of the sensitivity and specificity of the procedure in use is necessary. Abnormalities of pregnancy such as bleeding, hydatiform moles and choriocarcinoma, also require sensitive pregnancy tests. Hydatidiform mole and choriocarcinoma secrete large amounts of HCG[5] and require tests which are sensitive and in which HCG levels can be quantitated to distinguish them from normal pregnancy. When bleeding is due to abortion, the pregnancy test remains positive as long as active trophoblastic tissue is present, even if the foetus has been expelled[2]. It is therefore important to have tests which indicate even small amounts of active trophoblastic tissue present. Blood contamination of urine specimens from these patients results in the specimen being rejected because the presence of blood can give both false positive and negative results[3].

Haemagglutination-inhibition is the most sensitive means of detecting HCG, however, the reaction is only complete after two hours. It is also the method of choice for accurate quantitative results[1, 6].

There are two types of latex pregnancy tests, direct agglutination, and inhibition. Both are preformed on slides and the results obtained in two minutes. Sensitivity varies between manufacturers. Latex tests are generally not suitable for quantitative assays[2, 4].

Materials and Methods

Fifty urines and bloods were tested. The patients from whom the specimens were obtained, were grouped as follows:

(a) 5 non-pregnant females
(b) 5 normal males } —Control Group
(c) 6 normal pregnancies $< 8/52$
(d) 6 normal pregnancies $> 8/52$
(e) 28 other pregnancies.

Each urine had the following tests performed:

Specific gravity using a refractometer; Albustix for protein; routine microscopy and culture; 2 hour tube pregnancy test (Denco pregnancy test) and 1 minute slide pregnancy test (DAP test Macro), supplied by Smith Biolab Ltd.

Each serum sample had the following tests performed:

1 minute slide pregnancy test (DAP test Macro), Rheumatoid factor screen (Rheumaton) by Smith Biolab Ltd.

Results

All urines were free from protein, and their specific gravities ranged from 1.013 to 1.032. Nine of 50 urines showed leucocyte counts greater than 10×10^6 per litre of urine, 7 showed red cell contamination and 6 showed bacterial contamination (Table I).

All sera were negative for rheumatoid factor. With the exception of three patients in the control groups, none of the patients were on any medication at the time of specimen collection. Two of control group (a) were on oral contraceptives and one of group (b) was taking Indocid.

In control groups (a) and (b), there were no false positive slide or tube pregnancy tests, on either urine or serum (Table II).

In the normal pregnancy group (c), less than 8 weeks gestation, 5 of the 6 urines giving positive tube tests, gave positive slide tests. Two of these positive slide tests were very weak results. Three of six sera gave positive slide tests, with one giving a very weak result. Of these three positive sera, all had correspondingly positive urine slide and tube tests.

Group (d), normal pregnancy greater than 8 weeks gestation, all 6 patients had positive both serum and urine slide tests, and positive urine tube tests.

Group (e) contained patients with threatened or incomplete abortions at various stages of gestation from 6 to 20 weeks. Twenty-seven of the 28 urines gave positive slide tests, 5 of these 27 were very weak results. Slide tests on the sera showed 23 of the 28 sera were positive and 3 of these positives gave weak results. One of the negative slide test sera also gave a negative urinary slide test, but a positive urinary tube test.

Overall 2 of 40 urines gave false negative slide tests when compared to the urine tube tests. An additional 7 urine gave only weak positive slide tests and positive tubes. Eight of 40 sera gave false negative slide tests as

TABLE I

URINE RESULTS

Group	No.	SG Range	Leucocyte Counts $<10\times10^6$ per litre	Red Cell Counts $<10^6$ per litre Urine	Bacterial Contamination $<10^3$ organisms/litre	Positive Slide Preg. Test	Positive Tube Preg. Test
a & b	10	1.013-1.032	7	0	1	1	1
c	6	1.019-1.032	0	0	1	1	1
d	6	1.013-1.026	1	0	1	6	6
e	28	1.013-1.030	9	1	1	27	24

TABLE III

PREGNANCY TEST RESULTS

Group	No.	Positive Urine Slide Test (DAP)	Positive Urine Tube Test (Denco)	Positive Serum Slide Test (D.A.P.)
a & b	10	0	0	0
c	6	5	6	3
d	6	6	6	6
e	28	27	24	24

TABLE II

SERA RESULTS

Group	No.	Rh Factor Present	Positive Slide Pregnancy Test
a & b	10	0	0
c	6	0	3
d	6	0	6
e	28	0	24

compared to the urinary tube tests. Three other sera gave a weakly positive slide test with a positive urine tube test (Table III).

Discussion

In early pregnancy, the slide test gave false negative results with serum samples. The equivocal nature of the weak positive results obtained with both urine and serum is of concern if this test is to be used by people who would not normally read latex tests. Eight percent of the total sera and 14% of the total urines gave weak results with difficulty in interpreting whether truly positive.

Urinary contamination with blood, accounts for some pregnancy test urines being rejected[3]. A slide test on serum obviates the necessity for obtaining blood free urine, however, this is less sensitive than the standard Denco tube test and false negatives will occur.

Both slide and tube methods are simple to perform. The haemagglutination inhibition tube test takes two hours to complete and the direct latex test takes only one minute. No false positive urine or serum was found in this study of the modified DAP test kit, which has been introduced recently to New Zealand. A kit bearing the same name was previously withdrawn from the market because of false positive results[1, 6]. This problem was not experienced in the new kit.

Conclusion

A tube haemagglutination is required in the hospital laboratory to quantitate HCG levels in patients with choriocarcinoma or hydatidiform mole and to assist in distinguishing them from normal pregnancies. The tube haemagglutination inhibition test assists in the diagnosis of these conditions because of its greater sensitivity and should not be replaced in these situations. The latex slide method used in this trial can be used with either serum or urine and obviates the problem of obtaining blood free urine. A negative slide test on either serum or urine does not exclude the presence of trophoblastic tissue. False negative results may be due to either:

1. Very early pregnancy where the HCG level has not obtained a level high enough to give a positive slide pregnancy test, or
2. Where the HCG level has dropped below the sensitivity of the test for a number of reasons.

The introduction of the latex slide test as a screen on blood or urine for HCG levels would provide a rapid result which may in some cases, be helpful to clinicians.

Acknowledgments

My thanks to Smith Biolab. Ltd for donating the DAP Macro test kit used in this study.

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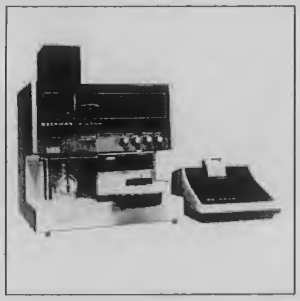

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The Identification of Drugs in Gastric Washings Following Acute Poisoning

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Received for publication, August, 1977

Summary

A method for the identification of drugs in gastric washings is described. Compounds of interest are extracted from the specimen by organic solvents, then subjected to U-V spectrophotometry, thin layer chromatography and, if desired, gas chromatography. The method is suitable for use in emergency situations and has been found to detect and identify the drugs commonly encountered in cases of overdose. Analytical time is between one and two hours depending on the number and variety of the drugs involved.

Introduction

In recent years the number of cases of acute poisoning seen at hospitals throughout New Zealand has been increasing[1]. This has resulted in biochemistry laboratories receiving many requests for analyses on specimens from the patients concerned. The type of specimen submitted includes gastric washings, urine and blood although occasionally some other sample is received.

Because many of the requests are made to smaller laboratories where drug and poison analyses do not justify a full time analyst, lack of experience may generate a reluctance to attempt this work. At Christchurch Hospital, when we are called upon to assist a laboratory by confirming their results, we have frequently identified drugs which are different from those originally reported. It is suggested that the problems some laboratories have in carrying out these analyses may be due to the lack of a readily available simple procedure.

The following method for identifying drugs in gastric aspirates has been in use in the Toxicology Section at Christchurch Hospital for many years. It has proved satisfactory in identifying most of the drugs encountered in overdoses (hypnotics, analgesics, sedatives, antidepressants, etc.), but should not be considered as being applicable under all conditions at all times.

Method

Figure 1 shows a flow chart for this procedure.

Make 20-25ml of gastric washings acidic (pH 1) with 1 mol/litre sulphuric acid and extract the mixture with 50ml of ethyl ether using a separating funnel. Remove the ether layer (by centrifuging first if necessary), dry with anhydrous sodium sulphate and filter it into a 100ml round bottomed Quickfit flask. An alternative method of drying the solvent is to filter it through a No. 1 PS Whatman phase separating filter paper. Return the extracted gastric washings to the separating funnel, make alkaline with ammonia and extract with 50ml of chloroform. Allow the chloroform layer to separate and then filter it into another 100ml round bottomed flask. For convenience, designate the extract from acid solution "A" and the extract from basic solution "B." The two are kept separate throughout the remainder of the analysis. Add a few drops of 0.1 mol/litre hydrochloric acid in methanol to "B" and evaporate both extracts to dryness on a rotary evaporator. Fatty residues will be obtained.

Dissolve these separately in 5ml of 0.05 mol/litre sulphuric acid using mild heat (waterbath) if required. Filter the solutions through No. 1 Whatman filter paper into glass stoppered 30-50ml tubes. Prepare a U-V spectrum of each of the clear filtrates over the range 200-450nm using 0.05 mol/litre sulphuric acid as a blank. Dilute both "A" and "B" as required to bring the whole spectrum on scale. Return all dilutions to their respective tubes.

Add sufficient concentrated ammonia solution to solution "B" to make it alkaline and then extract both "A" and "B" with 10ml

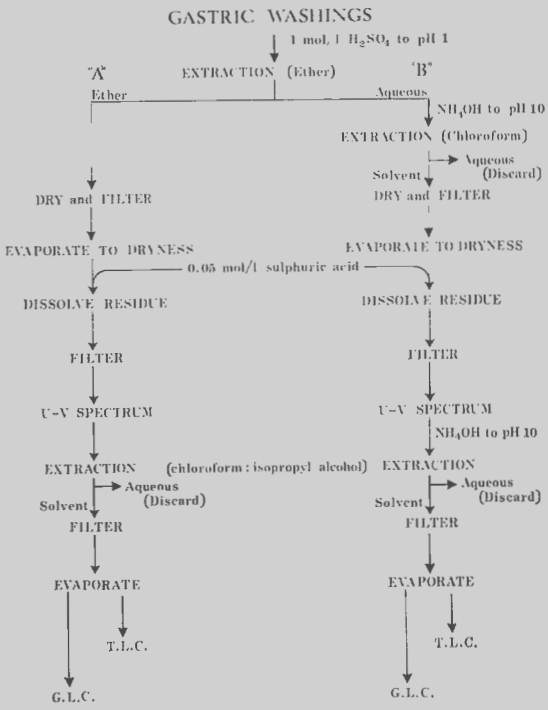

Figure 1. — Summary of analytical procedure used to extract drugs from gastric washings.

of chloroform: isopropyl alcohol (IPA) (9:1). Allow the phases to separate and remove the aqueous top layer with the aid of a water vacuum pump. Filter the remaining solvent layer through phase separating paper into a 25ml pear shaped Quickfit flasks or some other convenient vessel. Add two drops of 1 mol/litre hydrochloric acid in methanol to extract "B." Evaporate the contents of both flasks just to dryness, dissolve the residue in two drops of methanol.

Apply aliquots of both extracts onto silica gel plates together with any standards considered necessary as the result of the U-V spectra or information accompanying the specimen. Develop two plates in dichloromethane: ethyl acetate (6:1) and two or three in methanol: ammonia (100:1.5). When the chromatograms have run to about 75% of the plate height, remove them from the tanks and allow them to dry. Spray the dried plates with detection reagents to make the drugs present in the extracts visible. Table 1 gives a list of the drug types, the extracts in which they may be found, the solvent to use and the sprays useful in their detection.

The extracts remaining after the thin layer chromatography plates have been spotted are suitable for gas chromatography if desired. This can be carried out while the TLC plates are developing or left until the results from this step are assessed. Inject a portion of the sample into a gas chromatograph fitted with a flame ionisation detector and containing a column of 3% SE-30 as a stationary phase. Comparison with a reference compound or a pure sample of the suspected substance is used in the final identification of the drug.

Discussion

Extraction and U-V spectrophotometry

This part of the procedure is designed to rapidly extract most drugs from the gastric washing and to provide partial purification. The "A" fraction will contain the strongly acidic drugs like aspirin together with weakly acidic or near neutral compounds including the barbiturates, dilantin, paracetamol, diazepam, nitrazepam and methaqualone. The "B" fraction contains residual neutral drugs not fully removed in extract "A" and also the phenothiazines (e.g. chlørpromazine), antidepressants (amitriptyline), narcotic analgesics and the other basic drugs.

Fraction "B" is made acidic before evaporation to reduce the loss of amphetamine and the other anorectics by volatilization.

Solution of the fatty residue in dilute sulphuric acid serves a dual purpose. Most of the drugs encountered, including aspirin, the barbiturates and other acidic drugs are reasonably soluble in dilute acid while the fats in the residue are not. This gives a very convenient method of separating the drugs from the bulk of the remaining biological material. Secondly, dilute sulphuric acid is a recognised medium for the preparation of U-V spectra of drugs[1]. Lists of U-V peak maxima and drawings of spectra are available for individual drugs[1, 5]. However, it has proved convenient for us to prepare a library of spectra on our own spectrophotometer. This can be done for individual cases when a particular drug is suspected by acquiring a tablet of the material from the hospital pharmacy and preparing a reference spectrum. No interference from excipients has been found.

TABLE 1.

THIN LAYER CHROMATOGRAPHIC SYSTEMS USED IN THE IDENTIFICATION OF DRUGS.[d]

DRUG TYPE.[a]	TYPE.[b]	SOLVENT.[c]	SPRAY REAGENT.	COLOUR REACTION.
Salicylate	A	2	Ferric chloride.	Purple on a yellow background.
			Pauly Reagent.	Brown on a yellow background.
Paracetamol.	A & B	2	Pauly Reagent.	Brown on a yellow background.
Phenacetin.	A & B	2	Pauly Reagent.	No reaction.
Benzodiazepines.	A & B	2	Dragendorff's Reagent.	Orange on a yellow background.
Amitriptyline	B	1	Iodoplatinate.	Purple on a pink background.
			Marquis Reagent.	Range of colours depending on drug.
Imipramine	B	1	Iodoplatinate.	Purple on a pink background.
			Ammonium Vanadate - Sulphuric acid.	Blue on a yellow background.
Phenothiazines	B	1	Iodoplatinate.	Purple on a pink background.
			Marquis Reagent.	Range of colours depending on drug.
			F.E.N. Reagent.	Range of colours depending on drug.
Narcotic Analgesics	B	1	Iodoplatinate.	Purple to green on a pink background.
			Marquis Reagent.	Range of colours depending on drug.

a) Where a drug is named, other drugs of similar structure should also be considered.

b) "A" represents extraction from acidic solution and "B" represents extraction from basic solution.

c) Solvent 1; Methanol : ammonia (100 : 1.5).
Solvent 2; Dichloromethane : ethylacetate (6 : 1).

d) The list is by no means exhaustive. Reference should be made to publications dealing with this subject 1,2,5,6.

Such a collection can be built up progressively and maintained in a form convenient to the user. Alternatively a collection can be started in anticipation of its use. Such a compilation should at least include the benzodiazepines, antidepressants, phenothiazines, aspirin, paracetamol and any other drugs thought to be relevant. Reference to the U-V spectrum of a known pure drug and comparison with the trace obtained from a sample can save considerable time later in the analysis and makes the time taken preparing the library well spent.

The U-V spectrum is characteristic of the drug present. Unfortunately it is not necessarily unique and the presence of another U-V absorbing drug or interfering material will cause some modification of the trace. However, in most cases much useful information can be obtained and this is used as a guide for later steps. The spectrum may show early in the analysis whether or not large quantities of a drug are present. The shape of the spectrum will usually give an idea of the identity of the drug but should never be used as the only evidence of identification.

Thin Layer Chromatography

Thin layer chromatography is a valuable aid in the identification of drugs. By thoughtful use of R_f values and of sprays it is possible to make positive identification of individual drugs even in the presence of similar compounds. However, because of changes in humidity, temperature and solvent composition, care should be taken when comparing R_f values from TLC plates run at different times. Always run selected standards on the plates with the sample extracts.

The type of thin layer system used depends on the type of drug being sought. Clarke[1] recommends a range of thin layer chromatography systems for various drugs and poisons. A wide variety of basic drugs can be separated by methanol: ammonia (100:1.5) on silica gel and this solvent is very convenient to use. In our laboratory we have found dichloromethane: ethyl acetate (6:1) a very useful solvent for separation of many of the acidic and neutral drugs on silica gel. It can be expected that the majority of drugs found in overdoses will be separated by these two solvent systems. Special systems may be necessary in some cases,

for example in the separation of mixtures of sulphonamides or organophosphorus pesticides.

Because of the need to carry out thin layer chromatography rapidly, it has been found convenient to use small commercially coated fluorescent plates. These are available from E. Merck and Co., Damstad (5 × 10cm on a glass backing) and J. T. Baker Ltd, Phillipsburg, N. J. (2.5 × 7.5cm on a flexible backing). Both of these plates are sufficiently small to allow separations to be achieved quickly without the need to waste a large plate. They also provide a means of running several chromatograms at a reasonable cost.

The number of plates run in each solvent system will depend on the sprays to be used. It is important to set up at least one plate for the detection of barbiturates since this group of drugs does not give a satisfactory U-V spectrum in acid solution.

Various publications offer tables of TLC data[1, 2, 5].

Several systems have been collected by Clarke[1] together with the composition of many of the spray reagents needed. Sunshine[5] also gives an extensive range of TLC systems and reagents while Zweig and Sherma[6] is a useful reference for theory and for detector reagents.

Gas Liquid Chromatography

Gas chromatography has distinct advantages in confirming the identity of drugs isolated from samples. Conditions can be made reproducible from day to day which is something that cannot be said for thin layer chromatography. It is therefore possible to rely on the retention data particularly when used in conjunction with a reference compound. Tables of GLC data are to be found in a number of publications[1, 3]. These give retention times relative to reference compounds like codeine, diphenhydramine and ephedrine at various temperatures. By the use of these tables, or else from lists prepared by the user, it is possible to identify compounds when other information has given ambiguous or unclear results.

Gas liquid chromatography is a convenient method to use for the unexpected spot which may have appeared, for instance, when the thin layer plate was sprayed with Dragendorff's Reagent or some other non-specific spray. The presence of a peak at a particular retention time cannot necessarily be considered as proof of the presence of a given drug. This is because several drugs may have the same retention time under a particular set of conditions. In addition some substances will not lend themselves to gas chromatography because they are insufficiently volatile. All evidence accumulated in the course of the analysis should be taken into account when the final identification is being decided upon.

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It is intended to publish a further article dwelling on the use of other materials such as blood and urine for toxicological examinations and considering which is the appropriate material to use under different circumstances.—Editor.

A Simple Modification to Improve the Performance of an AAI Colorimeter

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Introduction

The first generation Technicon Colorimeter Module (AAI) introduced in 1955 has by now been superceded by the introduction of second and third generation Sequential Multiple Analysers (SMA) which process much of the routine work in the biochemistry laboratories of many hospitals However, the simple and

robust AAI colorimeter is still used for a variety of determinations that are not normally available on the SMA analysers, even though the AAI colorimeter lacks sensitivity at 340nm, is linear in transmission and possesses poor "wash" characteristics because of the long length of unsegmented stream flowing between the debubbler and flow cell. The introduction of the generation II flow cell with the debubbler situated very close to the optical path of the flow cell greatly improved the performance of continuous flow analysers. This deveopment was incorporated into the design of the SMA range of analysers and was one of the major advancements leading to the achievement of 100% steady state analyses at a rate of sixty samples per hour.

This communication describes how a conventional AAII flow cell can be fitted to an AAI colorimeter enabling the analysis rate of existing manifolds to be substantially increased without any loss in performance.

Instructions for modifying the flow cell cradle to accommodate the AAII flow cell

Reference in this section is made to the general operating instructions for the AAI colorimeter[1] and its accompanying Telephone Manual.

1. The AAI flow cell is removed from the colorimeter and discarded. The flow cell cradle can now be removed from the colorimeter chassis by unscrewing the adjusting nut (82CN) and releasing the set screw (86 CN).

2. The flow cell cradle (Fig. 1a) is now modified to house the AAII flow cell as shown in (Fig. 1b):

i The locating plate that faces the light source is unscrewed and discarded.

ii Section A is carefully cut away to accommodate the flow cell waste line.

iii The AAII flow cell 15mm × 2mm diameter (part No. 162—BO40) can now be positioned centrally at the rear of the flow cell cradle and the position of the locating peg (B) and threaded securing hole (C) marked for drilling.

iv A 1.5mm diameter hole is drilled at position B and a 10mm × 1.6mm (1/16 inch) diameter steel locating peg is pressed into this hole.

v A 2.35mm diameter hole is drilled at position C and tapped (UNC No. 4-40) to accommodate the knurled head captive screw of the AAII flow cell.

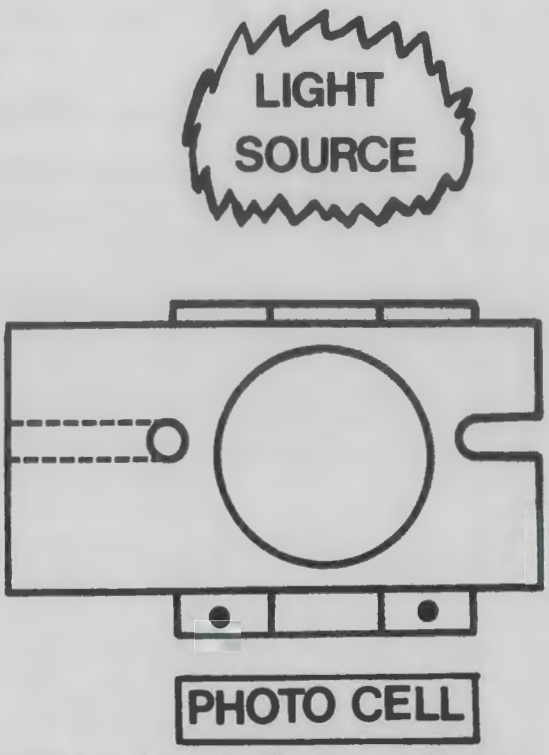

Fig. 1a. — Flow cell cradle prior to modification.

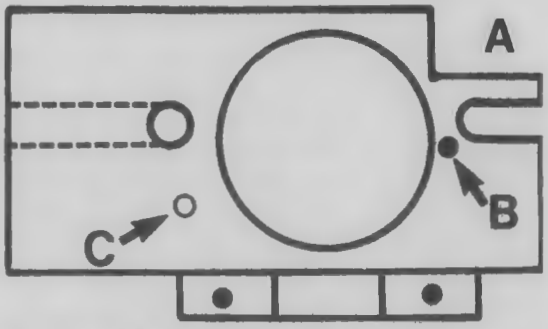

Fig. 1b. — Flow cell cradle after modification. A—Cut away to accommodate flow cell waste line. B—Flow cell locating peg. C—Threaded hole for knurled head captive screw of the AAII flow cell.

3. The modified flow cell cradle is now replaced in the colorimeter and is secured with the adjusting nut (82CN).

4. Three 300mm lengths of standard transmission tubing (part No. 116 0158 B01) are fitted to the AAII flow cell which is now

located into the flow cell cradle, securely fastened down with the knurled head captive screw and optically aligned[1].

Assessment of the performance of the modified colorimeter

The manifold chosen to assess the colorimeter was a urine creatinine manifold in which urine (0.1 ml/min) is sampled into air segmented (0.23 ml/min) glycine buffer pH 2.3 (1.0 ml/min), mixed (10 turn coil) and dialysed (300mm) against an air segmented (0.23 ml/min) water stream (1.0 ml/min). To the recipient stream on leaving the dialyser is added 50% saturated picric acid (0.23 ml/min) and 0.5 M sodium hydroxide (0.23 ml/min). This is mixed (30 turn coil) and the reaction is monitored at 505nm (flow cell return 1.0 ml/min). Using this manifold samples were run with the original AAI colorimeter and with the modified AAI colorimeter (Fig. 2). On comparison, the urine creatinine method when using the AAI colorimeter, operating at 60 samples per hour (60 2/1) achieves 91% steady state with 4% carryover, whereas using the modified AAI colorimeter the manifold operating at 80 samples per hour (80 6/1) achieves 99% steady state with only 1% carryover.

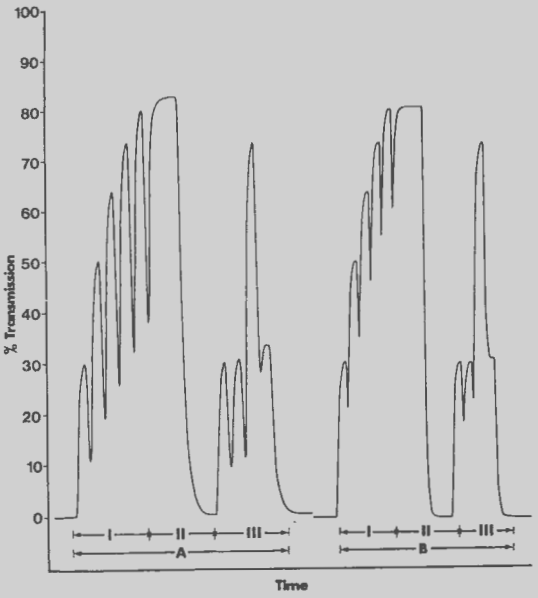

Fig. 2. — A—Urine creatinine manifold operating at 60 samples per hour (60 2/1) using the conventional AAI colorimeter. B—Urine creatinine manifold operating at 80 samples per hour (80 6/1) using the modified AAI colorimeter. I—Standard curve 0, 5, 10, 15, 20 and 25 mmol/l creatinine. II—% steady state experiment-extended sampling of 25 mmol/l creatinine. III—% carryover experiment 5, 5, 20 and 5 mmol/l creatinine.

Conclusion

The use of the modified AAI colorimeter module in place of the standard AAI colorimeter yields a valuable improvement in performance of continuous flow analysers in terms of higher percentage steady state, lower interaction between samples and increased rate of analysis with concomitant lower reagent consumption. This improvement can be achieved on existing manifolds employing AAI colorimeter modules by using readily available parts that are compatible with existing systems. The system described has operated satisfactorily over the past 12 months at Christchurch Hospital and the increased throughput of specimens has been greatly appreciated by the staff involved with the routine analyses.

REFERENCE

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The Nationwide Clinical Laboratory Computer System

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Member of the User Project Team since 1975.

Received for publication August, 1977. Updated

Introduction

The Nationwide Clinical Laboratory Computer System (NCLCS) is a data processing system for clinical laboratories. It is being developed by the Department of Health in conjunction with a User Project Team from all major hospitals.

The need for data processing systems in the larger clinical laboratories in New Zealand has developed as a result of automation, increasing workload, and the inability of existing staff to cope with this increasing workload. The systems which had developed prior to NCLCS were either reporting systems, involving key punching of test results into a computer system for reporting, or data acquisition systems which involve peak picking of test results from continuous flow analysers.

In both cases linking the patient identity information to the test results is not a computer function. Cumulative storage of test results is not a feature of either system.

NCLCS will utilise features of the existing systems in addition to others which a large computer system can support, notably a large centralised data base and rapid access to the Admission-Discharge patient information. On receipt of a patient specimen in a laboratory it is registered on line with the central computer and linked to patient information captured by the Admission/Discharge System. Work lists are produced by the computer. Manual results are entered via terminals in the laboratory. For automated tests, process control and data acquisition is performed by microprocessors and a minicomputer in the laboratory. In both cases data is transmitted by the network to the central system for further processing.

A variety of reporting formats, including cumulative result reporting, quality control, and management are printed in devices in the laboratory.

Development of D.P. Division

Difficulties experienced in hospital board data processing led the National EDP Committee in March 1974 to provide a report to the Department of Health.

This resulted in the employment of Touche Ross and Company, Management Consultants, to investigate the development of data processing in the health services. The Touche Ross report released in February 1975 recommended:

The establishment, as a division of the Department of Health, of a regionalised but centrally controlled data processing division.

The development of three "core" systems, covering three areas of hospital computing.

Admission and Discharge—Administration
Biochemistry —Scientific
Payroll System —Financial

The engagement of consultants as prime contractors for the implementation of their recommendations.

Touche Ross and Company were established as prime contractors in August 1975. Their immediate task was to establish the D.P. Division and develop the core systems.

The Data Processing Division of the Department of Health when fully established will have a permanent staff of about 120 to support the computer network of the Health Services. This organisation will act as a service bureau and will charge hospital boards for their use of the computer systems. There are two Regional Computer Centres, one situated in Auckland, and the other in Christchurch. Hospital boards south of and including Palmerston North will connect to the Christchurch Regional Computer Centre, whereas those north of Palmerston North will link to the Auckland Centre.

Associated with each hospital board area are Department of Health EDP co-ordinators who are responsible for the liaison between users of the system and Data Processing Division.

At each major hospital two mini-computers control the flow of data to the central system. A Transaction Terminal Cluster Controller (TTCC) which controls the flow of data from terminals, and the Remote Instrument Cluster Controller (RICC) which is used for process control and data acquisition from automated instruments. Data is transmitted by high speed leased Post Office lines or microwave bands to the central system.

Each laboratory will possess 6-10 terminal devices which include visual display units (VDU) and printers.

Project Development

The Project Team to design and implement the laboratory system was convened in November 1975. The scope of the initial "core" project was expanded to include Haematology because of the similar data processing problems experienced by the large automated Haematology laboratories. NCLCS will initially be implemented in the eight large hospitals because large automated laboratories show the greatest cost benefit.

The Project Team consists of a project leader, systems analysts and programmers, and 8 to 10 senior hospital laboratory staff. The latter have been seconded to the project, for 50 to 80% of their time, from the major hospital laboratories.

The Project Review Group was established to regularly review and constructively criticise the project throughout its course. The Review Group has wide representation from hospitals, private laboratories, universities and medical research institutions.

The User Requirements were completed in March 1976 and the System Specifications which represent the technical design of the system were completed in May 1977. This system will be implemented in three phases at the prototype site, Green Lane Hospital, Biochemistry and Haematology Laboratories.

After evaluation at the prototype site the complete NCLCS system is scheduled for implementation at the seven other largest hospitals in both Biochemistry, and Haematology, commencing June 1978.

Operation of NCLCS

The NCLCS workflow diagram, Figure I, shows the impact of the computer system on a laboratory.

Registration

Specimens and request forms received in the laboratory are compared and examined for completeness of data. The NCLCS system is dependent on all patients being assigned a Nationwide Patient number by the Admissions/Discharge system. Request forms accompanied by a patient number would have to satisfy certain minimum requirements regarding identification details before a search of the system is made to find the patient number. Request forms with insufficient identity details will be processed under a temporary patient number assigned by the laboratory system.

On receipt of specimens and forms in the laboratory, they are matched and examined for completeness. Computer generated specimen numbers incorporating check digits are attached. These are also used to label aliquots.

Specimen registration is initiated by keying in the patient number. After the patient information is displayed, the specimen number, specimen information, and request information is entered.

Batch Setup and Scheduling

Specimens are "queued" in the "process queue" with urgent samples first and in the order of registration. Batch lists are produced by the system and used to load the sample tray. Manipulation of batches is possible, using *on line* transactions.

Process Control and Verification

Process control of automated instruments is controlled by a special mini-computer called the Remote Instrument Cluster Controller (RICC). Terminals connected to it control and monitor the analytical process. Messages are produced and displayed to the operators when faults are detected during analysis.

Most existing laboratory instruments which produce analog signals will be interfaced to microprocessors. These devices for example, will be connected to Autoanalyzer colorimeters, will perform peak detection, analog to digital conversion, and transmit this signal to the RICC.

The RICC will perform standardisation, display the results to the operator for result verification, and transmit the verified results to the central system where they are passed to the patient result files.

Manual Result Entry

Other laboratory terminals not used for process control are connected to another mini-computer called the Transaction Terminal Cluster Controller (TTCC). Manual test results are "inputted" using terminals connected to this device. The calculated results are displayed to the operators to verify prior to passing into the patient result file. A high speed line printer is located adjacent to a TTCC where all result reports are printed at predetermined times.

Discrepancy Checking

At scheduled times of the day prior to report printing, the central computer system performs a number of checks on the validity of patient results. This process, called Discrepancy Checking will perform various checks on patient results to detect possible sample misidentification changes in patient condition, life threatening situations, or data entry errors.

These checks will eliminate the need for manually checking all reports, and significantly reduce the number of erroneous results reported by a laboratory.

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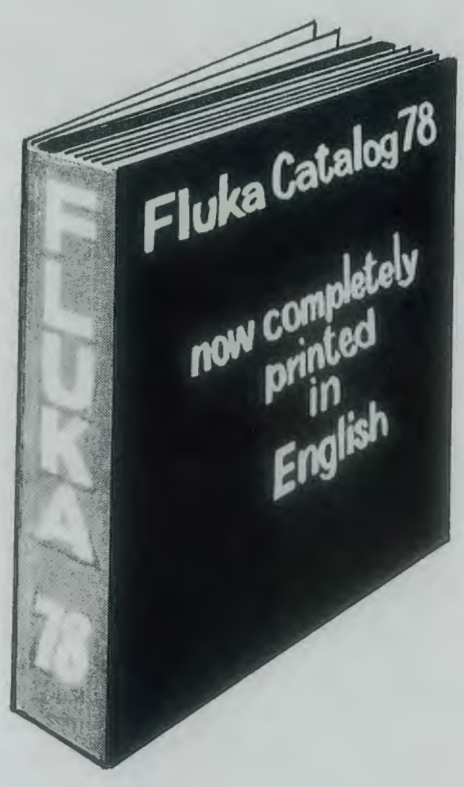

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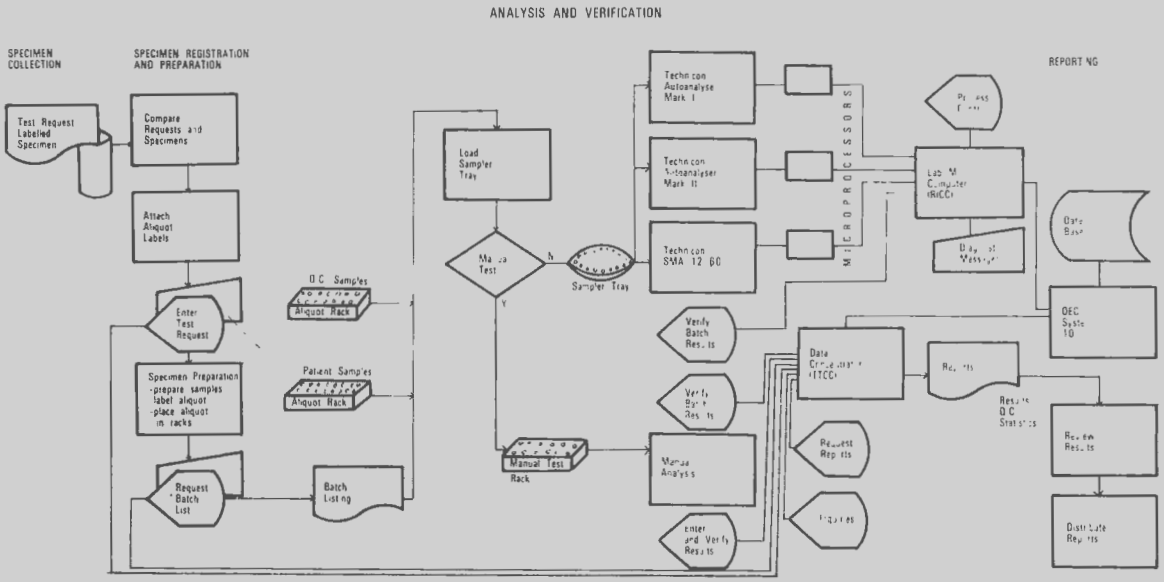

Figure 1. — NCLCS workflow diagram.

Result Reporting

The result reports produced by NCLCS will show those patient results which have been analytically verified and passed discrepancy checking. Various formats have been designed to support *cumulative* and *non-cumulative* reporting. The cumulative result reports show all results processed during the current admission for inpatients. For outpatients, cumulative reporting is performed as an overnight function. This is to enable the history files to be accessed in order to show previous outpatient visits on the same page. Cumulative reporting is not supported for patients who do not have a Nationwide Patient Number. Results will be maintained on line for the duration of the patient stay for inpatients, and from the time of registration to reporting for outpatients. In both cases, results will remain on the current file for seven days after processing. Figure 2 illustrates a cumulative report.

A cumulative index of all samples processed for the current year printed in alphabetic order by patient name, is printed weekly. The index points to specific volumes of Patient Result Books which show results cumulative for a week of any patient. The Index and Patient Result Books are printed weekly (overnight) and update previous versions. Multiple copies of the Index and Result Books can be obtained for distribution to the laboratory enquiry stations.

Enquiry

Access of the current file by the Current Patient Status Display will show all results processed during the current admission for inpatients, or sample processing period for outpatients. The status of the work in progress will also be shown. The Nationwide Patient Number is used to initiate the enquiry. To support telephone enquiries and provide a manual backup, the Index and Result Books will be the usual method. Specific searches of the archival tape file can be requested, but this search will be performed as an overnight function by the Regional Computer Centre. The report will be available from the hospital line printer the next morning.

Quality Control

Quality Control information is displayed at result verification to assist operators in assessing the results prior to passing them into the result files. Quality control information for a batch is summarised on the Batch Record which shows all test results, operator decisions at result verification, and quality control statistics for all quality control samples processed as part of the defined batch structure.

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TIME - 11.15 PAGE - 1

HAEMATOLOGY RESULTS

PATIENT	NAME		SEX	NUMBER	D.O.B.	LOCATION	TEAM
THORNBURY	MAXWELL	ALLAN	M	ADD1840	1JAN42		BROWN

SPEC.NO			98723-1	98826-4	99101-2	99116-3	99243-9
TYPE			VB	VB	VB	VB	VB
TIME			8.30	8.30	11.50	11.00	8.30
DATE			1DEC75	2DEC75	4DEC75	7DEC75	9DEC75
COMMENTS							
RBC	E12/1	4.5-6.5	4.6	5.8	5.9	6.1	6.5
Hb	g/l	140-180	150	141	144	143	148
PCV		0.40-0.54	0.48	0.49	0.51	0.50	0.52
MCV	fl	76-96	77	78	77	76	77
MCH	pg	27-32	33	31	34	39	40
MCHC	g/l	320-360	350	346	348	351	355
ESR	mm/h	3-5	4	1	3	4	1
RETICS	E9/l	24-84	25				
PLATELETS	E9/l	120-350					
LEUCOCYTES	E9/l	4.0-11.0	8.4			8.6	
BLAST	E9/l						
PROMYELOCY	E9/l						
MYELOCYTES	E9/l						
METAMYELOS	E9/l		4.45			0.86	
NEUTROPHIL	E9/l		2.10			5.68	
EOSINOPHIL	E9/l						
BASOPHILS	E9/l						
LYMPHOCYTE	E9/l		1.43			1.72	
MONOCYTES	E9/l		0.42			0.34	

98723-1 A SINGLE ERYTHROBLAST SEEN.THE NEUTROPHILS SHOW A SHIFT TOWARDS
1DEC75 IMMATURITY AND TOXIC GRANULATION

99116-3 THE NEUTROPHILS SHOW TOXIC GRANULATION
7DEC75

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Figure 2. — Example of a cumulative report.

Long term quality control is provided by monthly statistical and graphical reports. Various optional outputs can be obtained depending on the quality control specimen type. The charts include Precision, Levy Jennings and Cusum plots.

Management

Three reports can be obtained to assist the management requirements of a laboratory and provide the statistical information required by the Department of Health. The reports show analysis of workload processed by process group, the origin of the work requested and the distribution of workload over a 24 hour period.

Backup

In the event of component failure, specific backup procedures are used to allow processing to continue. Laboratory personnel will be trained to recognise component failures and take the appropriate course of action.

Staffing

All of the laboratory functions of NCLCS are intended to be carried out by existing staff except where large amounts of manual data are entered in which case data entry clerks may be required.

Discussion

The prototype development will take place at Green Lane Hospital, Auckland. The system will be implemented in phases, the complete system is scheduled to be in operation by mid-1978 in the Biochemistry and Haematology Laboratories.

After assessment of the prototype the full system will be implemented at the seven other hospitals:

Auckland Hospital
Christchurch Hospital
Dunedin Hospital
Middlemore Hospital
Palmerston North Hospital
Waikato Hospital
Wellington Hospital,

starting with Dunedin and Auckland Hospitals in mid-1978.

The development of NCLCS has provoked some controversy particularly relating to the following—

Teleprocessing

One of the major factors that will influence the implementation of NCLCS is the success of the computer network and transaction response times. Although teleprocessing is an accepted and successful technique for most applications there are many critics of this technique for laboratory data processing, and it is possible that as more systems utilise the existing Regional Computer Centres transaction response times may prove to be unacceptable. It is for this reason that the configuration of the RICC lends itself to expansion in that some critical functions, for example specimen registration, could in the future be off-loaded from the central system. The use of microprocessors for instrument control will also result in more efficient use of the RICC and has ensured that most existing laboratory equipment and new instruments with built in microprocessors can be interfaced.

Cost Benefit

The cost benefit analysis for a teleprocessing laboratory computer system proved cost justifiable for eight large hospitals in Biochemistry and Haematology. The initial operating cost of NCLCS, which does not include the development cost, has been shown to be $1.21 per specimen but it is unlikely that this full cost will be charged. Because the three core systems are the first to use the Data Processing Division the full charge will not be made until the Data Processing Division is fully operational.

The cost benefit analysis has shown that the tangible benefit such as reduction in clerical effort for worklist preparation, reading of test results, result transcription, and report preparation, will reduce total staff time by 16.8%. To achieve the benefit laboratories will therefore be required to reduce staff to produce this benefit. What is most likely to occur however, is that large automated laboratories which are currently experiencing a 10-20% p.a. increase in workload will be able to retain present staff levels. The computer system will enable them to absorb the increased demand for their services.

The intangible benefits of the computer system are considerable. Improved quality of results will result because of,

reduced turnaround time
more assured patient identity because of mandatory use of Patient Number
on line process control—error detection, and quality control
discrepancy checking
reduction in transcription, calibration, and calculation errors
increased processing capacity
collection of statistics by the system will enable more effective planning.

After all eight sites have been implemented the decision will be made as to whether further hospitals or additional laboratories within the eight will be implemented. This will depend on cost benefit but it is not expected that major design changes will be required.

Local Control

The permanent staff of the Data Processing Division will be responsible for the maintenance of the NCLCS hardware and software (programmes). It is envisaged that the system will undergo continual modification and improvement as a result of users' requirements that are cost justifiable. To most laboratory workers the Department of Health Computer System will have the "big brother" image because in the laboratory where hardware is installed strict control over the function of the equipment will be controlled by the Regional Computer Centre. This may prove frustrating

to enterprising laboratory scientists who as part of their present work continually modify and improve laboratory equipment and test methods. It is a pity that development of local Data Processing skills including programme development is not part of the Data Processing Division's policy.

The success of the centrally controlled computer system will depend on EDP co-ordinators who are both sympathetic and familiar with clinical laboratory functions.

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Technical Communication

Lancefield Streptococcal Grouping using an Autoclave, Pronase B Extraction, Counter-Electrophoresis Technique

From time to time the importance of Lancefield grouping beta haemolytic streptococci has been emphasised[4, 5] and while the Lancefield capillary tube method will traditionally remain the reference method, we have found the autoclave, Pronase B extraction, used in conjunction with counter-electrophoresis as developed by Edwards and Larsen[2], offers a system by which large numbers of beta haemolytic streptococci cultures can be rapidly grouped.

We have used a modified Edwards and Larsen method[2], as detailed below, for the past 18 months and have had good correlation with traditional methods.

Method

Two-four colonies of the suspected beta haemolytic streptococcus are subcultured from a Columbia Agar containing 5% human blood into 2ml sterile Todd Hewitt broth.

Incubate overnight at 35°C.

Autoclave culture tube at 15lb pressure for 15 minutes.

Cool tube.

Centrifuge for 5 minutes at 3000 rpm.

Decant supernatant broth.

Treat sediment with 0.2ml Pronase B solution containing 20mg Pronase B per ml prepared in Borate Buffer[1].

(Pronase B grade No. 53702 Calbiochem).

Incubate mixture for two hours in 37°C waterbath.

Centrifuge tube 5 minutes at 3000 rpm.

Retain clear supernatant, containing groupable streptococcal extract.

Counter electrophoresis is carried out using 0.05m barbital buffer, pH 8.6 (for both agarose and electrophoretic bath), at 7 MA/slide for one hour. Glass microslides are coated with 3ml of 1% agarose in barbital buffer. Wells 3mm diameter and 1.5mm apart are cut in agarose. 10μl streptococcal extract is placed in each well at cathode (negative) and group specific grouping serum in anode (positive) well.

Wellcome streptococcal antiserum ABCDG is used and precipitin lines are viewed with a hand lens using oblique lighting. It has been found necessary to leave slides to air dry approximately 15m to avoid missing a fine precipitin line when the agarose is moist. The biggest value of this extraction method combined with counter electrophoresis is that it enables this laboratory to provide a more comprehensive and rapid diagnostic service in Lancefield grouping all beta haemolytic streptococci.

S. W. Entwistle, ANZIMLT
D. A. Robertson, ANZIMLT
Pearson Laboratory,
Christchurch

June 1977

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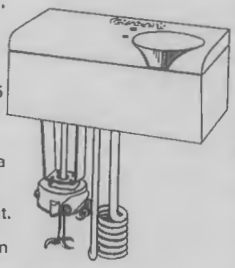

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ABSTRACTS OF PAPERS, NZIMLT CONFERENCE 1977

Biochemical Detection of Genetic Disease

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

Only during the last decade or so has it been technically possible to critically examine the basis for genetic disease. Present indications are that 25 to 30 percent of admissions to major children's hospitals in the developed countries are for genetic disease, congenital malformations, and/or mental retardation.

Details of work being undertaken in this unit for the detection of genetic disease, both ante- and post-natally will be presented. Particular reference will be made to possible environmental effects on the embryo and fetus in relationship to genetic disease.

The Use of Enzymes in the Diagnosis of Acute Myocardial Infarction

D. Dohrman, ANZIMLT
Waikato Hospital

Confirmation of the diagnosis of acute myocardial infarction depends on specific electrocardiograph changes and on alterations in the activity of serum enzymes and iso enzymes.

Forty-seven patients admitted to the coronary care unit with chest pain had the enzyme screen consisting of creatinine phosphokinase (CK), aspartate amino transferase (AST), alpha hydroxy butyric dehydrogenase (HBDH) and lactic dehydrogenase (LDH) together with the iso enzymes of CK and LDH on three consecutive days.

What contribution do these enzymes have in the diagnosis of acute myocardial infarction and is it necessary to measure all possible enzyme levels?

An Evaluation of the Enzymes Phosphohexose-Isomerase (PHI) and Gamma-glutamyl-transpeptidase (GGTP) in the Clinical Laboratory

G. Broad
Hamilton Medical Laboratory

This paper gives a brief introductory explanation of the enzymes, and then deals with the clinical usefulness of PHI and GGTP in the detection and monitoring of such disease processes as malignancy, liver disorders, etc.

Both enzymes are performed on a Centrifichem and have been in use for the past three years.

A Case of Primary Hyperoxaluria

D. J. Mikkelsen

Primary Hyperoxaluria is a rare hereditary disease resulting from a defect in the metabolism of oxalic acid in the body. Diagnosis of this disease rests upon the detection of increased levels of oxalic acid in the urine and demonstration of oxalosis of body tissues. A case of a six-year-old male with Primary Hyperoxaluria is presented together with details of laboratory investigations undertaken and suggested procedure for early detection of the disease in suspect cases. Laboratory differentiation of Type I and Type II variants is also discussed together with the metabolic defect associated with each type.

Physiology of Long Distance Marathon Runners

D. Reilly
Diagnostic Laboratory, Auckland

Eight athletes entered a marathon road race from Auckland to Wellington during November 1975. Samples were collected after each of the seven days and laboratory tests including: myocardial, liver, lipid protein, full blood count, and urinalysis screens were performed. These results showed at an early stage which athletes would complete the race and their eventual placings.

Muscular Enzyme Levels in Relation to Performance in Thoroughbred and Standardbred Racehorses

W. Wiggle
Diagnostic Laboratory, Auckland

The paper describes a pilot survey to study relative myocardial and skeletal muscle enzyme levels against performance under stress training and racing by racehorses over a season of racing.

Adaptation of "EMIT" Technique for Serum Diphenylhydantoin Assays to the Centrifichem System

R. Wilkes

I used a centrifugal analyser in a spectro-

photometric rate-measurement mode to determine the anticonvulsant drug diphenylhydantoin in serum, by use of a modified enzyme immunoassay ("Emit," Syva Corp.). I decreased reagent cost per determination. Also, the analysis rate is increased by measuring multiple samples simultaneously. My method requires only 10μl of serum for duplicate determinations. Accuracy and precision was excellent. Run to Run CV was better than 10%, and results for a series of samples compared favourably with results obtained by radio immunoassay ($r = 0.95$). The same method can be used for Phenobarbital, Primidone, Carbamazepine and Ethosuximide assays.

The Operation of a Wang 600 Calculator as a Data Logger for the SMA 12/60 Autoanalyser

E. M. Johnston
Department of Clinical Chemistry, Auckland Hospital

The SMA 12/60 Autoanalyser produces 720 separate results every hour of operation. Manual transcription of these results involves interpolation between marks on the recorder chart and hand-writing on to the worksheets for subsequent reporting. An unacceptable number of transcription errors occur with this operation and the machine requires one operator full-time recording results.

Automatic result recording in digital form has been accomplished by the use of a Wang 600-14 programmable calculator. The system utilises a retransmitting slidewire on the chart recorder together with a power supply and a Datel digital volt meter. The DVM is sampled by the Wang 600 via a Wang 605 interface. The programme allows for exclusion of off-scale values and spurious peaks due to short samples or air bubbles. Results are printed by the Wang 600 printer. The entire SMA 12/60 can be operated by one technologist and all clerical errors due to result transcription are eliminated.

Sequential Dialysis on the Technicon SMA 6/60 (4 + 2)

R. W. L. Siebers
Biochemistry Department, Wellington Hospital

Sequential dialysis by recycling the waste of each dialyser into the next one, plus the use of a common sample diluent, has reduced our sample size from 0.9ml to 0.4ml. This modification has resulted in the decreased use of pumptubes, reagents, calibrating and quality control sera, while maintaining precision and linearity for which results will be presented.

Fatty Acid Analysis of Amniotic Fluid

M. Legge
Perinatal Biochemistry Unit
Christchurch Women's Hospital

A rapid, quantitative method for fatty acid analysis has been developed which can be used for analysis of fatty acids from C-10 through to C-20.

This method is now in routine use for surfactant quantitation, replacing the L/S ratio for fetal lung maturation. The method is also being used to investigate possible significances of fatty acids in amniotic fluid.

Evaluation of the Pye Unicam AC 1 Automated Chemistry Analyser

R. Seibers

The Pye Unicam AC 1 system was assessed for accuracy and precision; and compared with methods from the Technicon Autoanalysers and the Vitation AKES. These results will be presented and discussed.

The Status of the Nationwide Laboratory Computer System

J. van Rijn

1. Why NCLCS?

Brief discussion of the objectives of NCLCS and the cost/benefit justification in terms of direct and indirect benefits.

2. What is NCLCS?

Commentary on the flow of work through a typical laboratory using the laboratory computer system. The major logical functions of the system and the most significant computer-produced reports will be related to this flow. These major functions to be followed through are:

:: Specimen registration
:: Batch setup
:: Test analysis
:: Enquiry
:: Control
:: Utilities

Some significant aspects of the system such as the provisions made for backup in the event of equipment failure will be commented upon. This will help to point out some aspects of the system as it now stands which represent changes from the original concept.

3. Current status of NCLCS

The implementation schedule will be briefly outlined.

4. Discussion

Some time is allocated to invite questions and general discussion.

A Case of Disseminated Intravascular Coagulation (DIC) Complicating Accidental Hypothermia and Associated with Pancreatitis, is Described

J. M. Mahood, M.A., MRCP (UK) and Anne Evans, ANZIMLT

DIC has been reported in association with many disorders (Wintrobe and others, 1974). Reports, so far, of DIC complicating hypothermia appear to be confined to infants (Chadd, Gray, 1972) and experimental animals (Johansson, Nilsson, 1964). This is to the best of our knowledge the first described case of hypothermia, DIC and pancreatitis occurring in an adult.

Normal Haematological Values in Childhood

R. Sheldon

Princess Mary Laboratory

A progress report of a study being carried out for normal haematological values with children of different age levels from infancy to teen. These values are being obtained with the Coulter ZF6 System.

Hemalog D

R. C. Sowden, ANZIMLT

An outline of equipment developed by the Technicon Corporation for the automation of White Cell Differential Counts.

Methods used for the identification of white cells will be discussed with interpretation of results obtained using these methods.

A Case of Penicillin-induced Haemolytic Anaemia

Teresa A. Signal

Immunohaematology Department, Dunedin Hospital

This patient was treated for a recurrent sub-acute bacterial endocarditis with high doses of penicillin. The diagnosis of haemolytic anaemia and other laboratory findings will be presented together with serological findings which suggested alternative treatment.

Factor XIII Inhibitors

Christine Hickton

A survey was caried out in which the plasma from 36 patients with malignant blood diseases was assayed for Factor XIII. This paper reports the results found and gives a short case history of two patients who were found to have inhibitors to Factor XIII.

Quantitation of Haemoglobin F

M. Legge

Perinatal Biochemistry Unit, Pathology Services, Christchurch Women's Hospital

A radioimmunoassay method is being developed for the quantitation of haemoglobin F. in adults. The method is simple and sensitive.

Using this technique haemoglobin F. is being quantitated during pregnancy as part of an investigation into the origin of fetal products in maternal blood. Details of the method and some anomalies of accurate haemoglobin F. quantitation will be discussed.

The Clinical Significance of Ferritin

Helen McLeod and G. W. Tisch

Recently, much interest has been shown in the serum level of the iron storage protein ferritin. Reported quantitation of serum ferritin has shown that levels tend to parallel the size of body iron stores in normal development and in cases of iron deficiency and iron overload.

The development of commercially available Radio Immunoassay techniques makes it possible to perform this assay in a Clinical Biochemistry laboratory equipped with a Gamma counter.

Ferritin is a protein of molecular weight 440,000 composed of 24 subunits. It has the ability to bind up to 4,500 iron atoms as a ferric hydroxy phosphate matrix.

Thus ferritin serves a two-fold purpose as it prevents toxicity by removing excess iron, and also provides a readily available iron reserve. Clinically it is of value in studying iron overload, anaemia, liver disease, malignant disease, porphyrias and in nutritional surveys. The ease in which ferritin can be quantitated makes it the method of choice over serum iron, total iron binding capacity and histological staining in assessing body iron stores.

Alcohol (ethanol) Produces more than Euphoria

J. Rees

Some abnormal laboratory parameters associated with excess alcohol intake.

An Unusual Case of Megaloblastic Anaemia

B. W. Main

Haematology Department, Dunedin Hospital

A severe case of megaloblastic anaemia will be described and illustrated which presented four months post delivery of a normal male child. The bone marow showed a virtual absence of erythropoiesis and some large primitive cells which gave rise to some concern for the correct diagnosis.

Laboratory Management of a Haemophiliac Undergoing Major Surgery

L. M. Milligan, Mrs S. Darling

A clinically, moderately severe young haemophiliac with recurrent haemarthroses of both knees, which led to a relatively severe incapacity of the right knee. The left had been deteriorating rapidly with more frequent haemarthroses, and a synovectomy was performed as a long-term measure to try to reduce haemarthroses and maintain joint mobility. A discussion concerning the laboratory management of this case will be presented.

A Case of Scurvy

Christine Hickton

Scurvy is a disease that is rarely considered possible in today's society. The name tends to make one think of sailing ships and lime drinks. However, it does still occur. In this case the diagnosis was suggested by the results of two tests, one of which is often omitted in the investigation of a patient's haemostatic function.

Packed Cells—Improved Quality and Viability

Miss J. Bryant

A new quad pack system was developed which in conjunction with citrate phosphate double dextrose anti-coagulant with an adenine enhanced electrolyte solution has improved storage potential (35 days) of packed cells. The biochemical parameters also compare favourably to other red cell packs.

Retinal Venous Occlusion and ABO Blood-groups

Lynette E. White

In this study 79 patients from the Opthalmology Department, Dunedin Hospital, with retinal venous occlusion had their ABO blood-groups determined. These were compared with those of blood donors in the Otago region.

Sixty-seven patients were followed up for at least six months after first attending. The visual outcomes of the affected eyes were related to the patients' ABO blood-groups.

A New Technique for Estimation of Red Cell Enzymes

C. S. Shepherd and G. E. Broad

Hamilton Medical Laboratory

This short paper discusses the use of automated equipment, in this case a Centrifichem, for the estimation of red cell enzymes.

Pyruvate kinase and Glucose-6-Phosphate Dehydrogenase estimations have been carried out by this method for about one year, using commercial kit set reagents. Because of the micro quantities used, there has been a considerable saving in reagent costs.

The method is quick, easy to do, has good reproducibility and is certainly sensitive to deficiencies of these enzymes.

It would be ideally suited for mass screenings of population groups, and we have found it a good method for our busy routine laboratory.

C3 Nephritic Factor

E. J. McKay

Immunology Department, Hamilton Medical Laboratory

West and Colleagues in 1965 described a proliferative type of glomerulonephritis with hypocomplementaemia. Spitzer and workers four years later identified a heat labile factor in the serum of patients with glomerulonephritis. This factor termed C3 Nephritic Factor—appeared to be a possible mechanism for persistently depressed complement levels observed in these patients.

C3 Nephritic Factor has now been shown to continually activate the complement system via the alternate pathway and results in prolonged cleavage of C3.

This brief will consider methods available for demonstrating C3 Nephritic Factor, the various diseases where it has been identified and the possible role it plays in disease.

Immunological Tests for the Diagnosis of Rheumatic Diseases

E. J. McKay

Immunology Department, Hamilton Medical Laboratory

The diagnosis of rheumatic diseases is generaly based on clinical, pathological and immunological criteria. Patients in this category often present with the clinical features of more than one disease therefore immunological tests play a major role in the precise classification of inflammatory joint diseases.

Immunological tests to be considered in this presentation will be the measurement of specific

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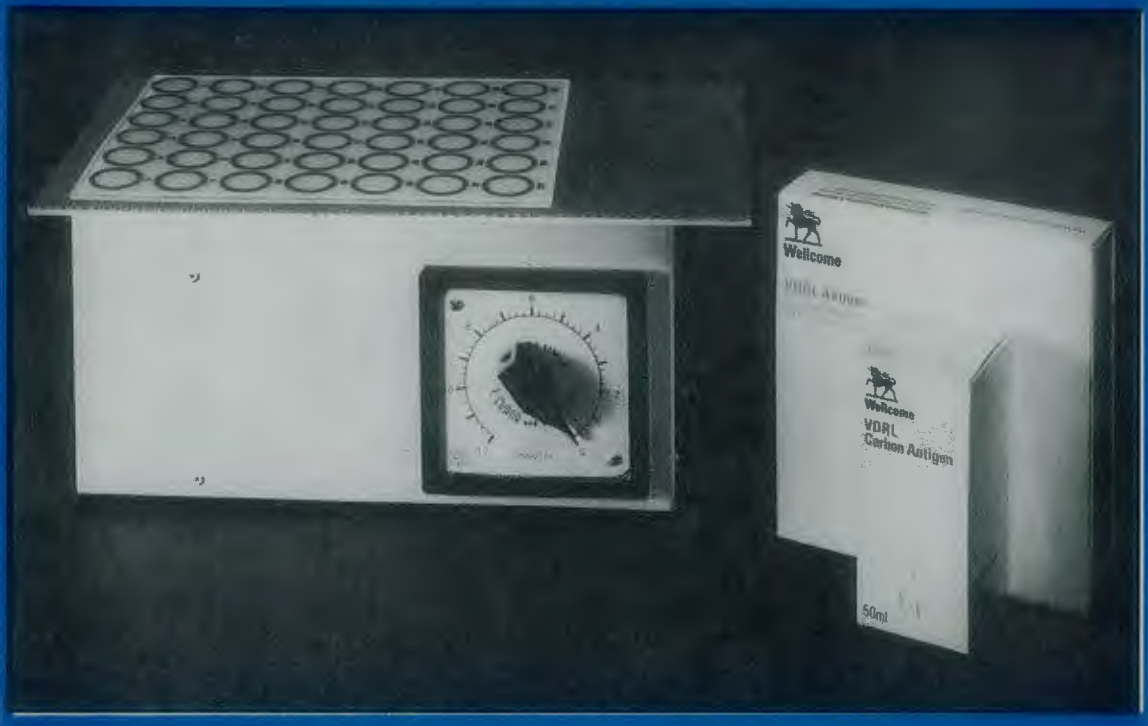

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and non-specific antibodies, the quantitation of certain plasma proteins, detection of immune complexes in serum and tissue and leucocyte function tests.

A Terminal Case of Tetanus

P. McLeod

It is intended to present this paper in two sections:

1. The Clinical Aspects
2. The Bacteriological Identification Procedures

The clinical aspects are of interest as they demonstrate the possibility of misinterpretation of tetanus rigor and spasm as a side effect to a drug which this psychiatric patient was receiving. These drugs will be covered briefly in this paper. At post mortem, the patient receiving no obvious causes for death except with the possibility of tetanus, which was suspected for two reasons . . . a badly infected finger and what appeared to be lockjaw.

The bacteriological identification procedures will be gone into with more depth. These will include initial isolation and purification procedures, biochemical identification and toxogenicity testing in mice.

For the cause of death to be listed as tetanus, it had to be proved bacteriologically. This places a lot of responsibility on a bacteriology department and it is hoped that this paper, with a discussion, may act as a refresher on this topic to those delegates attending.

The Preparation and Application of the Staphylococcal Co-agglutination Technique in the Routine Serological Identification of Bacteria

C. Curtis

The technique was applied to Streptococci, Enteropathogenic *E. coli*, Shigellae and Haemophilus. This method gives markedly superior reactions, allows combination of polyvalent antisera, and because of its increased sensitivity, antisera can be expanded 20 to 100 times.

The Gentamicin Antibody

P. McLeod

In presenting this paper I will be reporting on the findings of a project I have been undertaking for the past two years. My original intention was to develop a reversed indirect haemagglutination technique for the quantitative measurement of gentamicin in serum. This proposal involved the production of an antibody to gentamicin in rabbits and the purification of these antibodies by affinity chromatography. However, the haemagglutination reactions failed to perform as hoped—this probably being due to the nature of the antibody and its hapten, gentamicin. Precipitation tests have clearly shown that the antibody is specific to gentamicin and by its very nature of being a precipitating antibody other possibilities for using this antigentamicin-gentamicin complex are available to quantitatively measure this aminoglycoside.

This paper will be presented under the following headings:

1. Development of the gentamicin conjugate
2. Immunisation procedures
3. Purification by affinity chromatography
4. The nature of the gentamicin antibody
5. Future uses of the gentamicin antibody

At present a report of this project is being considered by a developmental laboratory in the United States of America. This is being arranged through Essex Laboratories Pty Ltd in Australia who is a subsidiary of the Schering Corporation. However, to date, I have yet to receive any further communication in regard to their assessment, but I expect to have received some comment before presenting this paper in Queenstown.

The National Serum Bank: Preliminary Results on Collections 1974-76

Margaret Berry

National Health Institute

The National Serum Bank is a collection of sera, stored at the National Health Institute, Wellington, consisting predominantly of samples taken from New Zealand donors. It is a joint New Zealand Blood Transfusion Service/Health Department ongoing project, designed to ascertain the changing levels of immunity to infectious diseases in the community. This paper discusses results of serological tests on the first three collections in Wellington (1974 and 1976) and Christchurch (1975), with respect to Toxoplasmosis, Brucellosis and Leptospirosis.

Minimum Protocols in Microbiology

A. Milne

It is evident from the work of the Co-ordinating Committee on Workload Units that many laboratories cannot understand the ap-

plication of the Canadian units, or else they are missing out very important steps in their handling of bacteriological specimens.

From these results:

Specimens	Mean Units	S.D.	Lowest	Highest
Blood culture	37.7	24.3	3	111
CSF	37.7	18.2	10	107
ENT Swabs	24.4	10.2	11	53
Faeces	35.5	16.2	6	71
Sputum	34.2	12.3	12	75
Urine	27.4	14.6	7	62
Wound Swabs	41.7	19.6	14	92

It is clear that gross errors of technique or accounting are common. We must attempt to standardise.

Candida parapsilosis Endocarditis

S. Gainsford, J. Fitchett
Microbiology Department, Wellington Hospital

In April 1977 a 55-year-old man was treated in the Accident and Emergency Department of Wellington Hospital for an infected left middle finger.

Five days later following a convulsion at home, he was admitted to hospital where a diagnosis of endocarditis was made.

Candida parapsilosis was isolated from his finger and blood cultures. This was tested for sensitivity to Amphotericin B and 5 Fluorocytosine singly and in combination.

An Evaluation of Sandiford's Malachite Green/Pyronin Counterstain for Gram Stains

Deborah Homan
Auckland Hospital

This stain should differentiate Gram negative bacteria from the background material, and should be of value in both direct smear, and blood culture examination.

This paper presents our findings at Auckland Hospital.

Diagnosis and Identification of the Aetiological Agents, of Primary Amoebic Meningo-encephalitis (PAM)

R. T. Cursons, T. J. Brown and Elizabeth Keys

Primary Amoebic Meningo-encephalitis is a fatal disease confined to the central nervous system and caused by free-living amoebae of the genera Naegleria and Acanthamoeba. To date there have been seven cases of the disease in New Zealand, all caused by *Naegleria fowleri* and all contracted after swimming in thermal pools. Diagnosis of Naegleria infection is accompanied by a recent history of swimming, an incubation period of 3-9 days in which nonspecific meningitis symptoms of headache, nausea, vomiting, stiff neck progressing to coma are expressed. Trophozoites are present in the CSF pathological features are a haemorrhagic necrotising meningoencephalitis with an acute inflammatory response, particularly at the base of the frontal lobes and the cerebellum which may contain many Naegleria amoebae. The portal of entry is the olfactory neuro epithelium.

Very much in the minority are cases of PAM caused by *Acanthamoeba spp.* all of which have occurred in chronic debilitated individuals or under immunosuppressive therapy. Diagnosis of Acanthamoeba infections is difficult due to the lack of specific symptoms and the absence of amoebae in the CSF. More informative findings may be revealed by the examination of throat and nasal swabs, or by serodiagnosis. Pathological features consist of a chronic, granulomatous encephalitis, the most affected areas being the midbrain and posterior fossa structures containing both trophozoites and cysts in the lesions. The portal of entry is thought to be the respiratory tract.

The identification of pathogenic free-living amoebae is further complicated by non-pathogenic species in both genera. *Naegleria spp.* can be differentiated from *Acanthamoeba spp.* on morphological, cytological, serological, physiological and growth characteristics as can the pathogenic and non-pathogenic species. The details of the identification strategy will be described.

We wish to acknowledge the financial support of the New Zealand Department of Health and the Medical Research Council.

An Investigation into the Stokes Method of Susceptibility Testing for Anaerobic Bacteria

Ina Te Wiata

The recommendations of the FDA for the correct technique to assess antimicrobial sensitivity to therapeutic agents using the methods outlined by Kirby-Bauer *et al* do not readily apply to the anaerobic group of organisms.

The commonest method of testing the susceptibility of organisms in this country is the Joan Stokes method.

This paper reports progress to date on an investigation to establish whether or not the Stokes method will give a reliable indication of susceptibility of these organisms.

Book Reviews

Dorland's Pocket Medical Dictionary. Twenty-second Edition, 1977. Published by W. B. Saunders Co. and obtained from N. M. Peryer Ltd, CPO Box 833, Christchurch. 741 pages, illustrated with colour plates. Price $N.Z.12.80.

How does one review a dictionary? Does it contain the "right" words?—and for who? This example of the medical lexicographer's art has a copyright going back to 1898, so it is plainly a successful publication. It undertakes to fulfil the three primary functions of a dictionary: the provision of spelling, pronunciation and meaning. It measures 18 × 13 × 2.5cm and contains roughly one-quarter of the material in the parent work, Dorland's Illustrated Medical Dictionary. This requires a good deal of selective pruning which must be a fairly arbitrary process. For example taking the first four syndromes in the larger work, Abercrombie's, Achord Thier's, Adair Dighton's and Adam Stoke's: only the latter appears in the pocket edition.

The preface states that thousands of new terms have been added and obsolete terms deleted. One would hope that new fields of medical knowledge would be catered for and indeed such words as tomography, radiotelemetry and radioimmunoassay could be picked out. Two pages are devoted to terms related to human immunoglobulins and words of current laboratory interest such as ferritin, osmolality, mucopolysaccharide, "fetoprotein" and fenfluramine (at random), appear.

Again a random search failed to reveal Crohn's disease or enzyme immunoassay.

The book contains a list of "combining forms" deriving the roots of the expressions used. There are a number of tables describing arteries, veins, bones, muscles, nerves and the chemical elements. There are illustrations of the fetus in various presentations, temperature equivalents, tables of weights and measurements, and sixteen very colourful anatomical plates.

What else can one say except, from the Laurence-Moon-Biedl syndrome good Lord deliver us!

R. D. Allan

Recent Advances in Blood Coagulation. Number 2, 1977. Edited by L. Poller. Multiple authors. 388 pages, illustrated. Price $N.Z.40.40 Published by Churchill, Livingstone and obtained from N. M. Peryer Ltd, Christchurch.

This is an excellent follow-up to the first printing of "Recent Advances," in 1969. Leon Poller is again assisted by 19 well-known contributors, in a rapidly published review that utilises references up to 1976. The speed of publication has resulted in a up-to-date review that, to a large extent, relieves the worker in this field of the heavy burden of trying to keep up with all the journals in recent years and the text is marred by only a few minor misprints.

The opening chapters on Biochemistry of Coagulation and related defence systems includes mention of Fitzgerald, Fletcher and Passovoy factors as well as recent work on structure and function on certain coagulation factors. The closely linked systems of coagulation, fibrinolysis, kallikrein/kinins and complement are discussed.

Chapter 3 is a physician-oriented approach to classification and diagnosis of bleeding disorders, and the two following sections deal with fibrinolysis, and natural inhibitors of coagulation (with emphasis on antithrombin III).

The current complexity of the F.VIII physiology/von Willebrand syndrome is covered in two chapters. The chapter on platelet disorders is somewhat disappointing with terms like "essential athrombia" leaving the reviewer cold.

In chapter 9 a new term is coined to describe the hypercoagulable state—"the Prothrombotic state" — and this "thrombosis predisposition state" is discussed under participatory forces, namely platelets, coagulation and fibrinolysis, red cells, blood flow and vessel wall. The possibilities (and limitations), of diagnostic tests are reviewed.

Poller contributes a chapter with a global view of coagulation abnormalities in liver disease. Recent developments in DIC are reviewed in Chapter 11, with a good section on management of the disease; the author (Cash) states his appraisal of heparin—"to

regard heparin as a potentially dangerous drug and reserve its use when all else is failing." I think this represents the mainstream view, and hope it will also clarify the opinions of examiners and examinees in our own Haematology syllabus.

The final chapters review dysfibrinogenaemias, the current therapeutic role of heparin, and coagulation problems in pregnancy.

This book should grace the shelf of all major coagulation units, especially those involved in post-NZCS teaching, in spite of its cost.

B. Rae

Review of Physiological Chemistry. H. A. Harper, V. W. Rodwell, P. A. Mayes and Associate Authors. Published Lange Medical Publications, California, 1977. 681 pages. Price $N.Z.18.20. Obtainable from N. M. Peryer Ltd, Christchurch.

The 16th edition of Review of Physiological Chemistry has been completely reorganised and two co-authors, Professors Victor Rodwell and Peter Mayes given recognition along with Harper The volume has increased in size from 570 pages to 681 and now contains 37 chapters compared with the 22 of the 15th edition.

The book begins with an introductory chapter containing a review of organic chemistry and the functional groups important in physiological chemistry. The material covered is principally however what was previously given in the appendix. This is followed by a chapter relating to the properties of water and its key role in the biochemical reactions of living cells.

The material on Enzymes, Hormones, the Gastrointestinal Tract, Water and Mineral Metabolism, Vitamins, Porphyrins, Calorimetry, Blood, Lymph and CSF and Epithelial. Connective and Nerve Tissues remains largely untouched. Muscle tissue however is now dealt with in a separate chapter and has been substantially altered and enlarged.

Amino acids and Peptides contain references to new techniques and there has been some rewriting of the existing material. Proteins have been allocated a separate chapter and the orders of protein structure dealt with in much greater depth. Biosynthesis of Amino acids contains three times as much material and is dealt with separately from Catabolism of Amino acids which remains unchanged.

The chapter on Carbohydrates is virtually unaltered but the chapter on Lipids includes a new section on the cell membranes and lipoproteins are dealt with more fully. A chapter has been added on the Regulation of Carbohydrate and Lipid Metabolism which contains a lot of new material, particularly with respect to the former. The section of the Citric Acid Cycle formerly dealt with in the chapter on Carbohydrate Metabolism has been largely rewritten and presented as a separate chapter.

Nucleotides and Nucleic Acids and Chromatin are now dealt with as two separate units—completely rewritten and updated in line with current knowledge. There is a large number of illustrations and the text is clear and concise. Similarly the chapter pertaining to Protein Synthesis and the Genetic Code has been rewritten and a chapter on the Regulation of Gene Expression is included.

Porphyrins contains an additional section dealing with jaundice, the estimation of serum bilirubin and the urobilinogen cycle. Immunoglobulins has increased from a brief mention in the 15th edition to a 14 page chapter dealing with basic details of immunoglobulin structure and techniques of quantitating. The text is clear and well illustrated.

Overall the book has been rearranged giving clearer divisions and headings which make reading easier. Updating of much of the material and presentation of new material has produced a volume substantially better than the 15th edition, and well worth purchasing either as a replacement or as a basic text for the uninitiated.

Janice Parker

NEW:
slide test for detection of antibodies to streptococcal exoenzymes

(an aid in detection of rheumatic fever and acute glomerulonephritis).

STREPTOZYME

Rapid : results in two minutes.
Easy : for titration simply dilute sample serially prior to test.
Self-contained : kit includes Reagent, Positive and Negative controls, glass slide and capillary tubes.
Sensitive : detects more specimens with elevated titers than any other single test. (1)
Specific : detects five related streptococcal enzyme antibodies* and is unaffected by nonspecific enzyme inhibitors. (2)
Stable : may be stored for over 1 year at refrigerator temperature.

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

* ASO, AH, ASK, ADNase, ANADase

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

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Index to Advertisers

Abstracts

Contributors: E. R. Crutch, Joan Dobson, Shirley Gainsford and L. M. Milligan.

CLINICAL BIOCHEMISTRY

Measurement of Serum and Plasma Ionic Calcium with the "Space-Stat 20" Ionized Calcium Analyser. Husdan, H., Leung, M., Oreopoulos, D. and Rapoport, A. (1977), *Clin. Chem.* **23,** 1775.

The authors found the Space-Stat 20 Ionized Calcium Analyser to be a convenient and reproducible instrument with advantages associated with printed-board electronic circuitry requiring no graph-calibration or involved calculations. Regular maintenance of this instrument is essential for "trouble-free" use. Limitations include the expense of its fluid pack and the cost and relatively short useful life of the present type calcium sensor.

—J. D.

Direct Potentiometric Measurement of Sodium and Potassium in Whole Blood. Ladenson, J. H. (1977), *Clin. Chem.* **23,** 1912.

The author compares results for sodium and potassium in whole blood and plasma as measured with a newly available potentiometric analyser, the "Orion SS-30." The lack of effect of protein and lipids on direct potentiometric measurements of sodium and potassium, and the capability for directly measuring these in whole blood make this approach faster and probably more accurate than flame photometry.

—J. D.

Inorganic Phosphorus Measurement: An Improved Method. Concustell, E., Cortes, M., Ferragut, A. and Gener, J. (1977). *Clin. Chim. Acta.* **81,** 267.

An improved micromethod for the determination of inorganic phosphorus in serum and urine is outlined. The procedure requires no deproteinization and yields a stable colour. The new formulation proposed avoids the pitfalls of other techniques. The method is both accurate (recovery 99-100.4%) and precise (c.v. 2.19%).

—J. D.

Accurate Photodensitometric Measurement of the Lecithin: Sphingomyelin Ratio After Elimination of the Acidic Phospholipids from Extracts of Amniotic Fluid Specimens. Gosselin, L. and Foidart, J. M. (1977), *Clin. Chim. Acta.* **81,** 209.

Treatment of human amniotic fluid lipids dissolved in a chloroform/methanol (9:1 V/V) mixture by batchwise addition of diethylaminoethyl cellulose in a dry state proved to be an easy and rapid procedure for the removal of the acidic phospholipids which may interfere in the photodensitometric evaluation of the lecithin-sphingomyelin ratio on a thin-layer chromotogram.

—J. D.

HAEMATOLOGY

Neutrophilic Hypersegmentation as an Indicator of Incipient Folic Acid Deficiency. Bills, T. and Spatz, L. (1977), *Am. J. clin. Pathol.* **68,** 263.

The authors have identified a group of subjects with neutrophilic hypersegmentation who are normal or near normal, with respect to other haematologic indices (haemoglobin, mean corpuscular volume). In a high proportion of these subjects, serum folate levels are abnormally low. The authors results indicate clearly that neutrophilic hypersegmentation may be a much more responsive indicator of tissue folate status than even erythrocyte folate levels.

—E. R. C.

Diagnosis of Alpha-Thalassaemia Trait from Coulter "S" Indices. Hedge, V. M., White, J. M., Hart, G. H. and Marsh, G. W. (1977), *J. clin. Path.* **30,** 884.

A number of patients of Mediterranean and Asian origins were found to have unexplained microcytic hypochromic red blood cells. Iron deficiency and Beta-Thalassaemia trait were excluded. The haematological indices of these patients, obtained on the Coulter model "S" were similar to those seen in obligatory heterozygotes for alpha-thalassaemia. Subsequent investigations showed that these patients had reduced rates of alpha-chain synthesis.

—E. R. C.

Disorders of Platelet Function. Hardisty, R. M. (1977), *Br. med. Bull.* **33, 207.**

This is one paper of 16 in this issue of the British Medical Bulletin dedicated to Haemostasis. In this paper, Hardisty gives an excellent review of platelet function, divided

primarily into hereditary and acquired disorders.

—E. R. C.

Factor VIII and its Inherited Disorders. Bloom, A. L. and Peake, I. R. (1977), *Br. med. Bull.* **33,** 219.

This excellent review covers the nature of Factor VIII, the physiological role of Factor VIII, the molecular variants of Factor VIII, carrier detection and Factor VIII antibodies.

—E. R. C.

Diagnosis and Management of Disseminated Intravascular Coagulation. Sharp, A. A. (1977), *Br. med. Bull.* **33,** 265.

Disseminated intravascular coagulation continues to present a challenge. This paper reviews the Pathogenisis, Pathology, Diagnosis and Management of DIC.

—E .R .C.

Normal Haemostatic Mechanisms: A Review. Pool, J. G. and edited by Gatlin, P. (1977), *Am. J. medical Technol.* **43,** 776.

This posthumus review by Judith Pool presents a relatively brief but succinct picture of current concepts, including the complex chain reaction, or multiplier effect involving intrinsic and extrinsic systems, the role of thrombin and the role of the liver.

—E. R. C.

Chromosomes in Haematology. Lawlor, S. D. (1977), *Br. J. Haemat.* **36,** 455.

The author, in this annotation, discusses the technical developments that have occurred since 1969, such as "banding" of chromosomes; discusses significant chromosomal associations and includes an excellent table of chromosomal abnormalities associated with haemopoietic disorders.

—E. R. C.

Cold Agglutinins in Infectious Mononucleosis and Heterophil - Antibody - Negative Mononucleosis-like Syndromes. Horowitz, C. A., Moulds, J., Henle, W., Henle, G., Polesky, H., Balfour, H. H., Schwartz, B. and Hoff, T. (1977), *Blood* **50,** 195.

Cold agglutinins were evaluated prospectively in patients with various mononucleosis syndromes and in a large control group. Cold agglutinins with anti-i specificity were seen mainly in heterophil-positive or negative Epstein-Barr virus-induced infectious mononucleosis.

—E .R. C.

Plasma Thromboplastin Antecedent (PTA, Factor XI): A Specific and Sensitive Radioimmunoassay. Saito, H. and Goldsmith (1977), *Blood* **50,** 377.

A specific, sensitive and reproducible radioimmunoassay for Factor XI has been developed with purified Factor XI and monospecific rabit antiserum. Precise measurements of PTA antigen were possible for concentrations as low as 0.3% of that in normal pooled plasma.

—E. R. C.

Haematological Changes Associated with the McLeod Phenotype of the Kell Blood Group System. Wimer, B. M., Marsh, W. L., Taswell, H. F. and Galey, W. R. (1977), *Br. J. Haemat.* **36,** 219.

The McLeod phenotype is inherited as an X-linked characteristic. The red cells have weak antigenicity in the Kell blood group and lack Kx, a precursor-like substance that appears to be necessary for proper biosynthesis of Kell antigens. Kx antigen is also required for establishement of normal cell morphology. Absence of Kx antigen causes a membrane abnormality in which the most prominent feature is acanthocytosis, and a compensated haemolytic state.

—E. R. C.

Evaluation of a New Anti-Fibrinogen-Coated Latex Particle Agglutination Test in the Measurement of Serum Fibrin Degradation Products. Marder, V. J., Cruz, G. D. and Schumer, B. R. (1977), *Thrombos. Haemostas. (Stuttg.)* **37,** 183.

A new latex particle test (FIBROTEX—Wampole Laboratories), has been compared with the Thrombo-Wellcotest and the tanned red cell haemagglutination inhibition immunoassay for sensitivity to purified fibrinogen and plasmic degradation products. These studies indicate that the new preparation, Fibrotex, is more sensitive and accurate in measuring FDP's than the Thrombo-Wellcotest. In addition, it is more reliable in tests with normal serum and urine, yielding fewer false positive results than the Thrombo-Wellcotest.

—E. R. C.

The Choice of Water for Coagulation Tests. Kaelin, A. C. and Andes, W. A. (1977), *Thrombos. Haemostas. (Stuttg.)* **37,** 570.

This communication is in the fo m of a letter to the editor pointing ou' the author's problems using deionized water rather than distilled water, for the performance of certain coagulation tests. A number of examples are given to illustrate the problems.

—E. R. C.

IMMUNOHAEMATOLOGY

Anti Tn and Anti-Cad in the serum of the non-poisonous male Python sebae. Lockyer, W. J., Gold, E. R. and Bind, W. E. (1977), *Med. lab. Sciences* **34,** 4.

Separable anti-Cad and anti-Tn have been found in the serum of non-poisonous Python sebae. Anti-Cad reacted only with protease treated red cells. Immunological and biochemical tests were compared.

—L. M. M.

Classical Pathway Complement Actuation in Association with Paraproteinaemia. Naish, P. F., Collins, C. and Barratt, J. (1977), *Immunology* **33,** 4.

Classical pathway complement actuation was found in a number of patients with paraproteinaemia. The mechanisms of hypocomplementaemia proposed for some of the patients are cryoglobinaemia and *in vivo* immunoglobulin aggregation.

—L. M. M.

The Introduction of Adenine Fortified Blood Preservatives. Swisher, S. N. (1977), *Transfusion* **17,** 4.

This article discusses the background to the use of Adenine in fortified blood preservatives and covers some of the research and problems involved.

—L. M. M.

Adenine in Blood Banking. Simon, E. R. (1977), *Transfusion* **17,** 4.

Adenine supplementation of ACD and CPD preservative media may permit prolonged red blood cell storage shelf life and increase the quality of the stored red blood cells. Its use could increase the flexibility and efficiency of blood transfusion services.

—L. M. M.

MICROBIOLOGY

Counterimmunoelectrophoresis in the Diagnosis of Bacterial Meningitis. Golding, Hanne and Lind, Inga. (1977), *J. clin. Mic.* **5,** 405.

In addition to microscopy and culture, counterimmunoelectrophoresis (CIE) was performed with antisera to Neisseria meningitidis (groups A, B, C), Streptococcus pneumoniae (omniserum and pools A to I) and Haemophilus influenzae type b. on 283 cerebrospinal fluids. Although CIE was positive in only 57% of specimens which were culture positive to these three organisms, it was positive in 12% of culture negative specimens. It is pointed out that direct microscopy must also be done as four CSFs contained Escherichia coli, three of which reacted with N. meningitidis group B antisera and one with H. influenzae type b.

—S. G.

Selective and Enhanced Recovery of Group A and B Streptococci from Throat Cultures with Sheep Blood Agar containing Sulphamethoxazole and Trimethoprim. Gunn, B. A., Ohashi, D. K., Gaydos, Charlotte, A. and Holt, Edith S. (1977), *J. clin. Mic.* **5,** 650.

Sheep blood agar containing 23.75 μg of sulphamethoxazole and 1.25 μg of trimethoprim per ml. (SXT-BA) was compared with sheep blood agar (SBA) for isolating group A and B streptococci from throat swabs. In the routine laboratory SXT-BA recovered 28% more group A and 37% more group B streptococci than did SBA, mainly due to the suppression of "viridans" streptococci.

—S. G.

Serological Typing of Pseudomonas Aeruginosa: Use of Commercial Antisera and Live Antigens. Brokopp, C. D., Gomez-Lus, R. and Farmer, J. J. III (1977), *J. clin. Mic.* **5,** 640.

A slide agglutination test using Difco commercial antisera and live antigens from a 24 hour culture is used to serotype Pseudomonas aeruginosa strains. 93.2% were typable with live antigens. Using heated antigens as a comparison 94.5% were typed and when both antigens were used 96.3% were typable. The reproducibility and specificity of the method were examined and its application for studying outbreaks of Pseudomonas aeruginosa infection is shown.

—S. G.

Specific Direct Fluorescent Antibody Detection of Treponema Pallidum. Daniels, Katie C. and Ferneyhough, Hope S. (1977), *Health lab. Sci.* **14,** 164.

Specific Direct FA staining of T. pallidum (DFATP) in lesion exudate was compared with darkfield microscopy and the FTA ABS test on patients' sera, to determine its reliability as a diagnostic test. The DFATP was as reliable as darkfield microscopy and had some advantages. It is stressed that reliable results depend upon good specimen collection and training in fluorescent antibody techniques.

—S. G.

Stability of Working Reagents for the Modified Rapid Fermentation Test (MRFT). Brown, W. J. (1977), *Health lab. Sci.* **14,** 172.

The MRFT for Neisseria gonorrhoeae is a sensitive and quick test. The reagents of the MRFT (buffered salt solution and carbohydrate) were mixed then tested for their stability at –70C, –20C, 4C and 22C over a six month period. Another improvement in the method was made by omitting the making of a suspension of N. gonorrhoeae and directly inoculating the fermentation tubes.

—S. G.

Modified Bacteriological Swabs for the Transport of Anaerobes in Clinical Specimens. Smith, Lynda L. and Ferguson, I. R. (1977), *Med. lab. Sci.* **34,** 247.

Qualitative studies of gram-negative non-sporing anaerobic bacilli showed that survival was prolonged on heated blood agar swabs when exposed to atmospheric oxygen. Quantitative studies then showed that the red cell and haemoglobin were the major factors in prolonged survival so haemoglobin alginate swabs were used in a clinical study. These showed a much better recovery of anaerobes from clinical specimens after 24 hours exposure to oxygen than serum albumin swabs and were easy to prepare, autoclavable and stable.

—S. G.

A Comparison of Three Rapid Methods for the Detection of B Lactamase Activity in Haemophilus Influenzae. Skinner, A. and Wise, R. (1977), *J. clin. Path.* **30,** 1030.

This paper gives the methods for the phenol red, chromogenic cephalosporin, and iodometric tests for the rapid detection of B lactamase activity in Haemophilus influenzae. When compared, all methods gave the same results but the chromogenic cephalosporin method was the most rapid to perform and its reagents were the most stable lasting three weeks at 4C.

—S. G.

Haemophilus Vaginalis (Corynebacterium Vaginale): Method for Isolation and Rapid Biochemical Identification. Greenwood, J. R., Pickett, M. J., Martin, W. J. and Mack, E. G. (1977), *Health lab. Sci.* **14,** 102.

This paper presents a method for the isolation of Haemophilus vaginalis on a medium which allows it to be distinguished from vaginal diphtheroids and lactobacilli. Rapid biochemical identification based on the Buffered Single Substrate test for Neisseria gonorrhoeae.

—S. G.

Antibodies and the Aberdeen Typhoid Outbreak of 1964: I and II. Brodie, J. (1977), *J. Hyg.* **79,** 161.

Part I presents and compares the results of Widal tests carried out on a group of TAB immunised healthy individuals and typhoid fever patients from the Aberdeen typhoid outbreak in 1964. One factor that emerged was that the response to the somatic antigens was poor and often delayed and the flagellar antibody titre was a more reliable aid in diagnosis. Vi agglutinin titres as high as 1:40 were found in healthy individuals with no evidence of enteric fever or TAB inoculation. Part II evaluates the Coombs test, complement fixation and fimbrial agglutination tests as possible alternatives to the Widal.

—S. G.

Biochemical Typing of Urinary Escherichia Coli Strains by Means of the API 20 E Enterobacteriaceae System. Davies, B. I. (1977), *J. med. Micro.* **10,** 293.

574 strains of E. coli isolated from patients with urinary tract infections were serotyped and biotype using the API 20 E Enterobacteriaceae system. Of the 55 different biotypes identified, two accounted for 42% of the strains and seven others accounted for between 8.4 and 1.9%. There was little correlation between biotyping and serotyping.

—S. G.