JULY, 1981

ISSN 0028-8349

THE NEW ZEALAND JOURNAL OF

medical laboratory technology

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

VOLUME 35, No. 2

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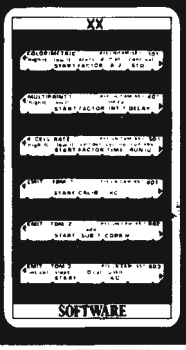

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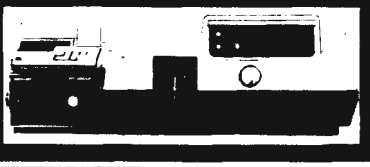

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THE NEW ZEALAND JOURNAL OF

Medical Laboratory Technology

Vol. 35, No. 2 July, 1981

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

Subscription to the JOURNAL for non-members is NINE DOLLARS per year or THREE DOLLARS FIFTY CENTS per single issue, postage paid. Overseas subscription rates on application.

Intending contributors should submit their material to the Editor, C/o Immuno-haematology Department, P.O. Box 946, Public Hospital, Dunedin, New Zealand. Acceptance is at the discretion of the Editor, and no undertaking is given that any article will be published in a particular issue. The copy deadline for each issue is the first of the month prior to the month of publication.

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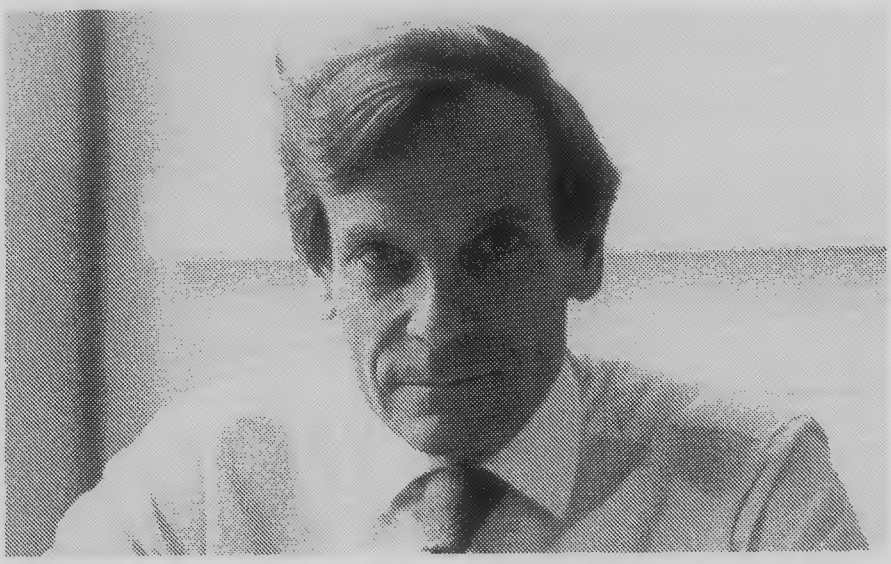
Ortho Diagnostic Systems Visiting Lecturer 1981 Mr. Laurie Marsh



Mr. Laurie Marsh, the distinguished Immunohaematologist from the New York Blood Centre, will visit Australia and New Zealand during August and September as the Ortho Diagnostic Systems 1981 Visiting Lecturer.

During his visit he will address topics such as auto-immune haemolytic anaemia, the genetics and function of blood group antigens, quality control in automated serology, null phenotypes, and cold auto agglutinins.

Meeting dates and locations are:

August 13 — Red Cross Blood Transfusion Service – Brisbane, Qld.

August 15 — Orange Base Hospital – Orange, N.S.W.

August 17 — Red Cross Blood Transfusion Service – Sydney, N.S.W.

August 19-21 — Australian Institute of Medical Laboratory Scientists, Annual Scientific Meeting – Melbourne, Vic.

August 24 — Australian Society of Blood Transfusion, Annual Scientific Meeting – Adelaide, S.A.

August 25-28 — Royal College of Pathologists of Australasia, Annual Scientific Meeting – Adelaide, S.A.

September 2-4, New Zealand — Institute of Medical Laboratory Technologists Annual Scientific Meeting – Wellington, New Zealand.

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Quinicrine Banding (Q-Bands) of Human Chromosomes

D. R. Romain
Cytogenetic Laboratory, Laboratory Services, Wellington Hospital, Wellington, New Zealand
Received for publication 23 March 1981

Summary

This paper describes techniques for the study of Q-bands, Q polymorphisms and Y chromatin (Y body). The methods can be applied to human chromosomes from cultures of venous blood, marrow, fibroblast and amniotic cell cultures.

Introduction

It was clear from the middle sixties that a breakthrough into the identification of human chromosomes other than by conventional and auto-radiographic techniques was sorely needed. The answer came in a series of papers published from 1968 to 1972 by a group from the Karolinska Institute, Stockholm, led by T. Caspersson, Caspersson *et al.* (1968)[1], (1969a)[2], (1970a)[3], (1970b)[4], (1970d)[5], (1971a)[6], (1971b)[7], (1971e)[8], (1971f)[9], (1972)[10].

The experiments began with the staining of plant and hamster chromosomes with a fluorescent dye—quinicrine mustard. Viewed under ultra-violet (UV) illuminated microscope it was soon observed that each chromosome showed a constant distribution of negative and positive fluorescence. Spurred on by these findings, attention was then turned to human chromosomes, where it was likewise found that each chromosome had a specific banding pattern of its own, consistent from person-to-person, and for all types of tissues (Figure 1).

The breakthrough then had arrived; a whole new era of chromosome identification had been ushered in. An explosion of publications followed as the technique was applied to medicine, old syndromes were updated and new ones brought to the fore, a new age had dawned in clinical cytogenetics.

Materials and Methods

Method for Q-bands of metaphases from venous blood, marrow, fibroblast and amniotic cell cultures.

Solutions and Reagents

1. 0.5 percent (weight/volume) Quinicrine dihydrochloride (Atebrin) in glass distilled water.
2. MacIlvaine's buffer pH 5.5

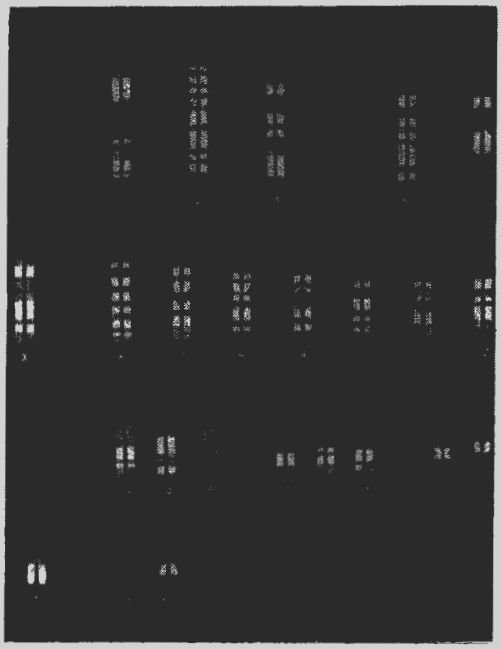

Figure 1.—Ideogram of human genome showing main Q-bands.

Procedure

1. Slides are flooded with 0.5 percent aqueous quinicrine dihydrochloride solution for 10 minutes on a staining rack.
2. Differentiate in gently running tap water for one-three minutes.
3. Rinse in distilled water and blot dry.
4. Mount in distilled water pH 5.5 or in MacIlvaine's buffer pH 5.5
5. Seal edges of coverslip with rubber cement solution.

Notes on Procedure

1. When mounting slides use the minimum of mounting media possible, avoid air bubbles.
2. Rubber cement solution is preferred to nail varnish or wax for sealing coverslip, since it can be removed to permit restaining or other banding procedures.
3. The quinicrine dihydrochloride solution keeps for up to six weeks with no apparent deterioration, and appears to work best three days from time of preparation. Store in a darkened cupboard.

4. Chromosome preparations that have been stored for several months or even years can still produce good bands if the pH is adjusted accordingly. Lowering the pH will result in brighter fluorescence, but bands may be less defined. Raising the pH will improve band definition, but reduces the brightness of the fluorescence, and there is a tendency for the chromosomes to swell up and deteriorate when exposed to u-v light. Therefore, for older preparations, it will be necessary to experiment. In our experience, a pH of 4.0-4.4 seems to give satisfactory results.
5. Some workers prefer to refix slides in methanol before step 1 of the procedure, but we find this not to be necessary.
6. This method is also applicable to preparations that have been destained after conventional analysis. Preparations which have been mounted in D.P.X. and then destained will not produce good results.

Method for Y chromatin (Y body) examination in amnion cells, interphase nuclei of lymphocytes, polymorphs, fibroblasts and oral mucosa cells.

Solutions and Reagents: as for Q banding

Procedure
1. Slides are flooded with 0.5 percent aqueous quinicrine dihydrochloride for seven minutes on a staining rack.
2. Differentiate in gently running tap water for three-five minutes.
3. Rinse in distilled water and blot dry.
4. Mount in distilled water pH 5.5, or McIlvaine's buffer pH 5.5.
5. Seal edges of coverslip with rubber cement solution.

Notes on Procedure
1. Differentiation is longer than for Q-bands. Insufficient differentiation will obscure bright fluorescent body from surrounding cell chromatin.

Preparation of Cells for Y chromatin (Y body) analysis.
1. Blood films for polymorph analysis: Make slides as for normal white cell differential, fix in methanol for five minutes on a staining rack, allow to dry in air, then proceed.
2. *Oral mucosa cells:* Make slides as for Barr Body analysis fixing wet in 50/50 alcohol/ether. Bring down to water through graded alcohols, then proceed.
3. *Interphase nuclei of lymphocytes:* Use slides that have been prepared as for chromosomal analysis.
4. *Uncultured amniotic cells:* A drop of the amniotic fluid cell pellet is placed on a grease-free glass slide and allowed to air dry. Fix in methanol or methanol/acetic acid 3:1 for five minutes, allow to air dry. Continue with procedure.
5. Amniotic cells that have not attached during first seven days of culture: Centrifuge into a cell pellet and remove supernatant. Resuspend cells in dregs of supernatant and then add methanol/acetic acid 3:1 fixative solution. Leave to fix for 20 minutes. Centrifuge and resuspend cells in fresh fixative. Centrifuge and remove supernatant. Resuspend in dregs of supernatant and place cells on a grease-free glass slide and allow to dry in air. Continue with procedure.
6. *Fibroblasts:* Use slides that have been prepared as for chromosomal analysis.

Microscopy and Photography

All work in this laboratory is carried out on a Zeiss photomicroscope III, with fluorescent attachment. The microscope is equipped with a HBO 50 W super pressure mercury lamp and filter sets Cat. No. 487706 and 487709. Only two objectives are used: A Neofluar 16/0.40 for scanning, and a Planoapo 100/1.3 oil immersion for photography and detailed cell examination.

Photography is performed using a Kodak Panatomic-X film with printing being done on Ilfobrom glossy single weight paper 4.1P.

Results and Discussion

Except for minor differences, such as the centromeric and secondary construction regions of chromosomes 1, 16, 3 and the distal portion of the long arm of the Y chromosome, the Q-bands correspond with the G-band pattern, the brightly fluorescent regions corresponding with Giemsa positive bands and the negative fluorescent regions corresponding with Giemsa negative bands.

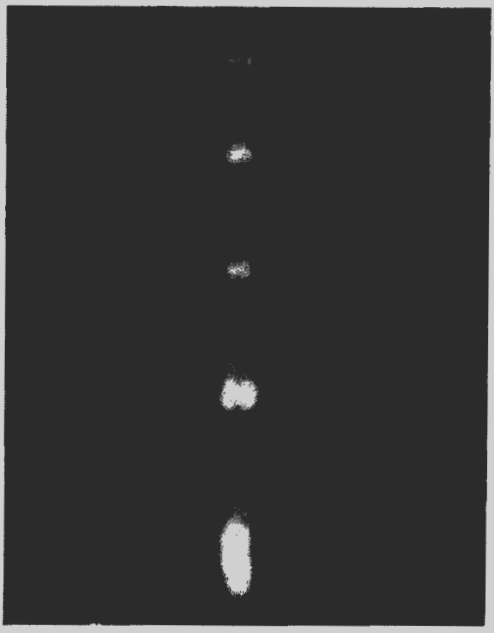

Figure 2.—Polymorphisms of the distal long arm of the Y chromosome.

The Y chromosome, because of its distinctive brightly fluorescent distal segment of the long arm, was the first chromosome to be identified with quinicrine fluorescence, Zech (1969)[15]. Studies have shown that in the population, a few phenotypically normal males exhibit no brilliant segment at all, and that there is a wide variation in the size of the Y chromosome due entirely to the variation in the length of the distal segment (Figure 2).

Because of this polymorphism, the Y chromatin or Y body also exhibits variation in size.

Due to the presence of other very brightly fluorescent polymorphic regions in the human genome, such as the centromeric region of chromosomes 3, 4, 13 and the satellites of the acrocentrics (Figure 3), misinterpretation as to the positive report of Y bodies can occur.

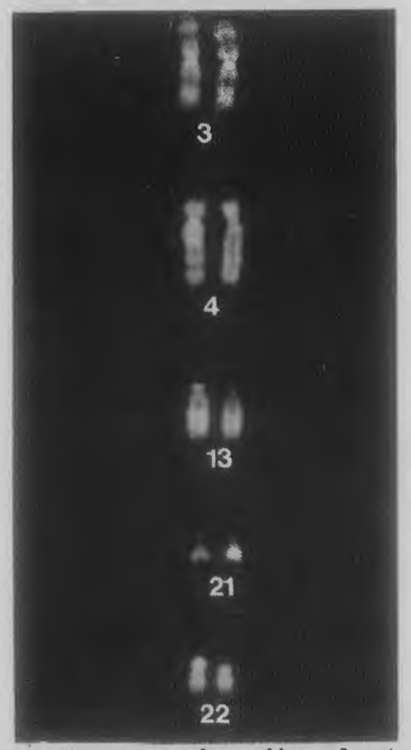

Figure 3.—Fluorescent polymorphisms of centromeric region of chromosomes 3, 4, 13 and satellite regions of chromosomes 21 and 22.

Fluorescent Y bodies can be demonstrated in a variety of cells: fibroblasts, interphase nuclei or lymphocytes, oral mucosa, spermatozoa, amnion cells, polymorphs and hair roots, Pearson *et al.* (1970)[12], Polani and Mutton (1971)[13], Francois *et al.* (1971)[11], Sumner *et al.* (1971)[14] (Figure 4).

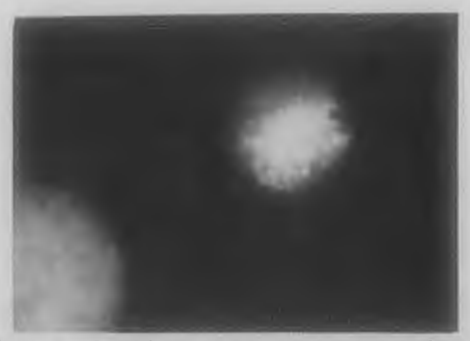

Figure 4.—Interphase nucleus of lymphocyte from a 47XYY patient showing 2 Y bodies.

Generally speaking, however, the experienced cytogeneticist is able to be discerning in this matter of false positives and negatives, especially in prenatal sexing, by examining parental karyotypes and carrying out Barr Body studies.

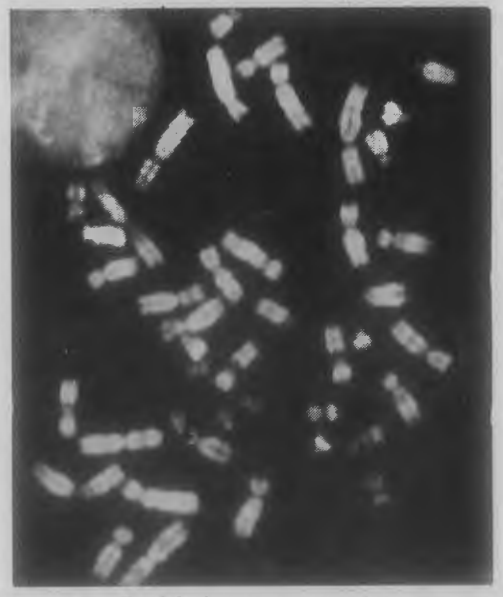

Figure 5.—Partial karyotype showing the 2 Y chromosomes in a 47 XYY patient.

With regard to the morphological variants observed in the human genome mentioned previously, their size and fluorescent intensity are a constant inherited feature and have no pathological effect—indeed, they have proved to be valuable markers for genetic analyses. As with G, C and R-bands, the Q-bands have proved to be of immense importance in the identification of chromosomal aberrations (Figures 5 and 6). The principal disadvantage of Q-banding is the

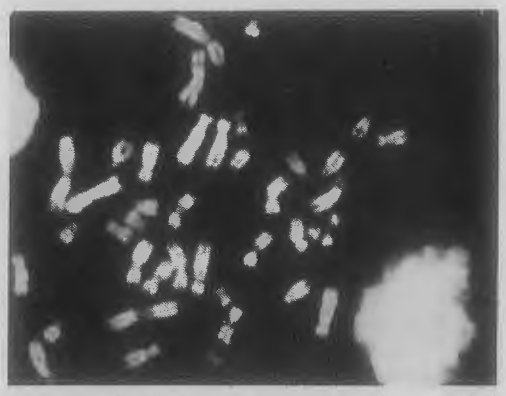

Figure 6.—Partial karyotype showing the 3 brightly fluorescent 21s in Down's syndrome. Note 21s are in satellite attraction.

temporary, short-lived nature of the fluorescence and the expense of the fluorescent microscope set up; nevertheless, no regional cytogenetic centre can afford to be without such a useful diagnostic tool in this ever-expanding scientific field.

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Parasitic Zoonoses in New Zealand

P. F. L. Boreham

Queensland Institute of Medical Research, Bramston Terrace, Herston, Brisbane, Queensland, Australia 4006

Received for publication 1 April 1981

Paper presented to the 36th Annual Conference of the New Zealand Institute of Medical Laboratory Technology, 20-22 August 1980.

Introduction

Zoonoses are those diseases and infections which are naturally transmitted between vertebrate animals and man. Many of the world's most important parasitic diseases are zoonoses and they may involve (a) farm animals e.g. cysticercosis, trichinosis, (b) domestic pets e.g. toxoplasmosis, hydatid disease or (c) wild animals e.g. trypanosomiasis, leishmaniasis. Zoonotic diseases exert a two-fold effect on a community. Firstly they may result in disease both in humans and in animals causing morbidity and often exacerbating protein—energy malnutrition. Secondly, economic loss may result through (a) reduced capacity of draught animals to work effectively and (b) meat being unsuitable for consumption or export. For example, in 1956 the export of meat from New Zealand to Britain was threatened because 10% of the cattle livers being imported by Britain were found to contain deep seated cysts of *Echinococcus granulosus* (Anonymous 1957).[5]

Little is known of the parasitic diseases of New Zealand before the arrival of the Europeans. Andrews (1976)[3] suggested that only two

Table 1

Zoonotic Parasitic Diseases Endemic in New Zealand

	Species	Vertebrate Host	Transmission to Man
Protozoa	*Toxoplasma gondii*	Cat, dog, sheep and other vertebrates	Transplacental transfer Contaminative routes Ingestion of cysts
Nematoda	*Gongylonema pulchrum*	Ruminants, pigs, bears, hedgehogs, monkeys	Ingestion of cock-roaches or dung beetles
	Toxocara canis	dog	Ingestion of eggs
	Trichinella spiralis	Pig, rat, dog, cat, wild carnivores, bears	Ingestion of under-cooked meat
Cestoda	*Echinococcus granulosus*	Dog, sheep, cattle, horse, camel	Ingestion of eggs
Trematoda	*Cercaria longicauda*	*Aythya novaeseelandiae*	Snail intermediate host. Active penetration of cercariae

endoparasites were present in the Maoris before 1769, "Iro" or thread-worm (*Enterobius vermicularis*) and "Ngoiro", probably *Ascaris lumbricoides*. The six zoonotic diseases now endemic in New Zealand are thus almost certainly recent introductions (Table 1 adapted from Andrews 1976).[4]

Toxoplasma gondii (Nicolle and Manceaux 1908)

It is only recently that the complete life cycle of this ubiquitous parasite has been elucidated and its association with cats revealed (Hutchinson *et al.* 1971).[14] Serological evidence suggests that it is common among the human population of New Zealand. Using the dye test, Manning and Reid (1956)[17] showed that the incidence rose from 27% in the 16-25 year age group to 65% in those persons over 56 years. Limited data would suggest that sheep farmers and abattoir workers, especially those handling carcasses, have higher incidences of antibodies to *T. gondii* than the general population (Nuttal 1967).[21] The actual incidence of disease caused by *T. gondii* in New Zealand is unknown. Congenital toxoplasmosis (Reid 1956; Weston and Bush 1962),[23, 23] lymphadenopathy (McCreanor 1962)[19] and cases of myocardial involvement (Adams 1962)[1] have all been reported. The exact mechanism by which humans become infected is not understood but is probably due to ingestion of oocysts from cats faeces. Certainly transplacental transfer of organisms occurs and various contaminative routes, including eating undercooked meat are suspected of being important. Cysts are known to be common in the brain of infected animals. Droplet infection and spread by flies are other possible routes, especially as herbivores and carnivores are often infected to similar degrees. Although cats are known to pass oocysts in their faeces, which become infective for man after approximately 48 hours when sporulation has occurred, their exact role in transmission is still uncertain.

Gongylonema pulchrum (Molin 1857)

The gullet worm is an extremely rare human parasite and under 50 cases have been reported worldwide. It is normally a parasite of ruminants with various species of dung beetle and cockroach acting as intermediate hosts. Infection of the final host occurs by ingestion of the infected insect. Adults and maturing larvae may be found migrating through the mucosa and submucosa of the buccal cavity of man where irritative change may result. A single human case has been reported from New Zealand (Johnston 1936).[15] This was a person of Jugoslav origin who had not left New Zealand during the previous two years. He consulted his physician stating that a week before he had felt something in his throat and that the object had gradually made its way forward to the mucosa of his upper lip. No other symptoms were reported. A live worm was eventually extracted with a pin and identified as *Gongylonema*

pulchrum. How this person acquired the infection is uncertain although cockroach contaminated food or water was suspected.

Toxocara canis (Werner 1782)

The dog roundworm *T. canis* has a direct life cycle. Eggs require two-four weeks at 15-30°C and a relative humidity above 85% to embryonate in soil. Man is infected by accidental ingestion of embryonated eggs. The larvae hatch and migrate throughout the tissues but do not develop into adults. Hence the common name, visceral larval migrans. The most serious pathological effect occurs if the larvae accidentally invade the eye and granulomas form. Puppies present the major risk to human health as they become infected prenatally and the majority of animals less than six months of age in New Zealand are infected (Charleston 1977).[11] Although human cases have been reported (Becroft 1963, 1964; Frankish 1965)[6, 7, 13] the prevalence of infection in man is unknown. In a seven year period only one case of visceral toxocariasis was seen in a large children's hospital with an annual through-put of 4-5000 children (Charleston 1977).[11] Many cases could well go undetected because of the difficulties of diagnosis and the mildness of symptoms in some patients. The recently developed ELISA test using secretory antigens (de Savingy *et al.* 1979)[12] should improve diagnosis.

Contaminated soil is another source of infection. Ova were recovered from 24% of 800 soil samples collected from public places throughout Great Britain (Borg and Woodruff 1973)[8] and present a reservoir for infection. The risk however is minor when compared with physical contact of children with infected puppies. Effective control may be achieved by regular deworming of puppies (prenatal infections have been shown to become patent 23 days after birth) and also bitches after parturition since they may also be passing eggs in their faeces.

Trichinella spiralis (Owen 1935)

Man is infected by eating undercooked meat, commonly pork, containing muscle cysts. Three human cases of trichinosis have been reported from New Zealand (Mason 1978).[18] The first occurred in 1926 in Christchurch and was probably acquired in France during World War 1. The two other cases were acquired locally, in Wellington in 1931 and Auckland in 1964. Following the Auckland case, local pigs, cats and rats were found infected and subsequently infected rats were also found at Feilding, North Island and infected cats and pigs at Burnside near Otago. It seems probable that wild cats and rats maintain small foci of *T. spiralis* which occasionally infect pigs, the human cases resulting from eating undercooked pork. In New Zealand the disease is extremely rare and is best controlled by adequate surveillance of pigs in abattoirs.

Echinococcus granulosus (Batsch 1786)

Hydatid disease caused by *E. granulosus* is the most important and serious zoonotic disease in New Zealand. It is essentially a parasite of dogs with sheep as the intermediate host. The parasite is not specific and many other herbivores may also ingest eggs on pasture and become infected. If man accidentally ingests eggs the larval stages develop (protoscolices) within the hydatid cyst, usually in the liver or lungs. The history of the disease from 1878 has been documented by Burridge *et al.* (1977).[10] There was a steady increase in prevalence from 28.7 per million population for the period 1878-1881 to 77.2 per million for the period 1945-1954, when New Zealand had one of the highest prevalence rates in the world. Since that time there has been a dramatic fall in the incidence of this disease so that only five human cases were reported in 1979 (Unpublished figures, Department of Health). The successful control of hydatid disease was largely achieved through an education programme backed up by legislation. The major features were the setting up of voluntary hydatid committees, prohibiting the feeding of raw offal to dogs and instituting regular arecoline purges of dogs to check for tapeworms.

The highest incidence of hydatid disease was found along the East Coast of the North Island, which is a sheep farming area, with a high Maori population (Burridge and Schwabe 1977).[9] These authors showed that the relative risk of hydatid infection among Maoris was 6.4 times greater than among Europeans. This high incidence rate among Maoris appears to be due to their system of land tenure which results in small blocks of land which are uneconomical to farm. A second important factor is that Maoris, unlike Europeans, tend not to discriminate between working dogs and pets, so that their animals are allowed into living areas after having contact with sheep. Thus cultural, as well as behavioural factors, have been responsible for the high incidence among the Maoris. The successful control of hydatid disease in New Zealand has served as a model for other areas such as Tasmania.

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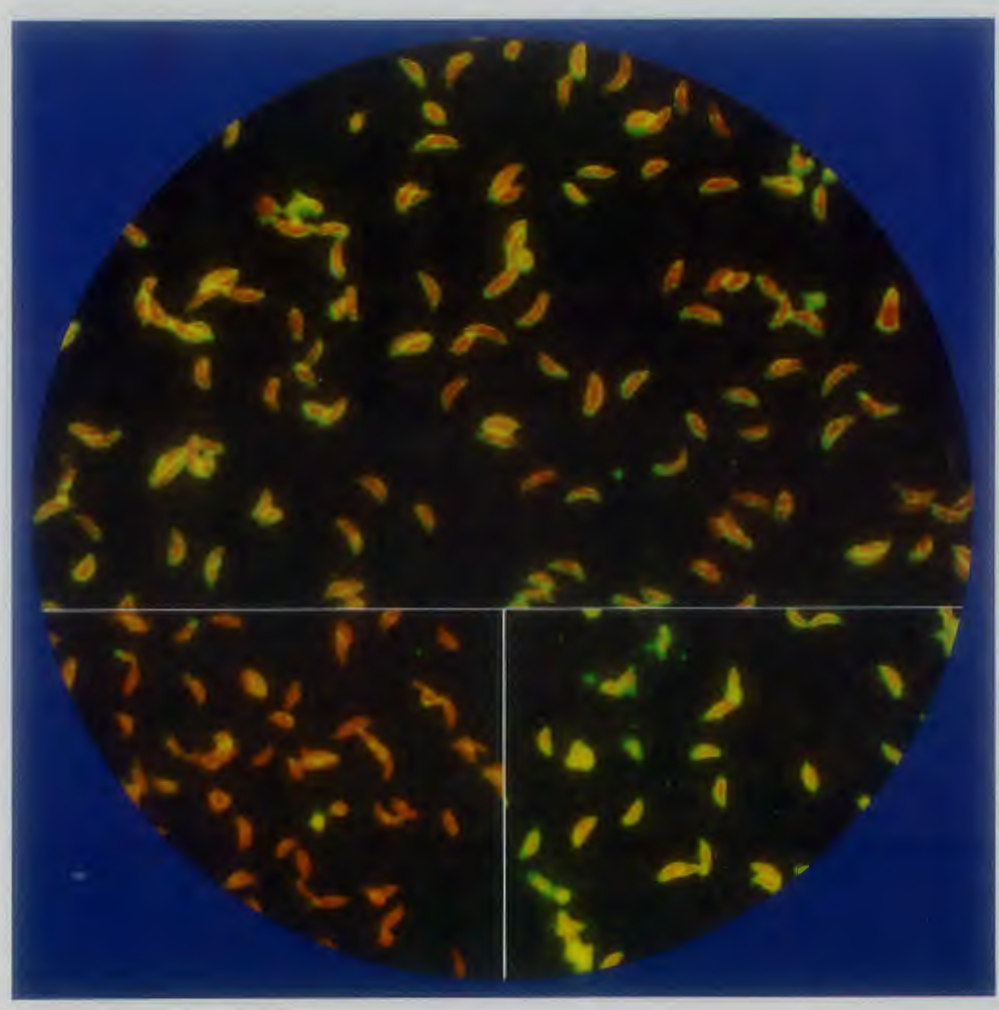

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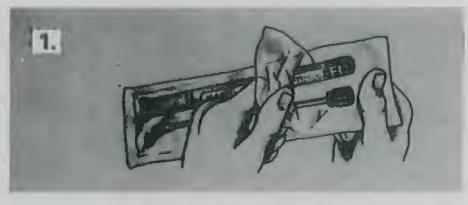

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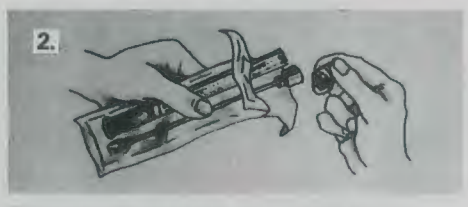

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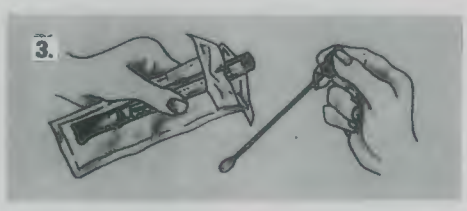

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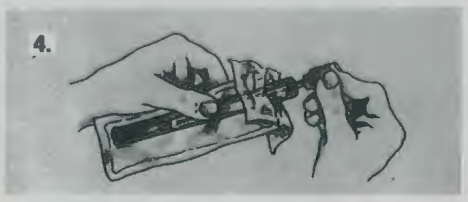

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Cercaria longicauda (MacFarlane 1949)

The cercariae of many species of mammalian and avian schistosomes will penetrate human skin but not develop any further. If the person has been previously exposed to the parasite and is sensitised, a hypersensitivity reaction resulting in a dermatitis occurs. This is often referred to as "swimmers" or "bathers" itch. MacFarlane (1949)[16] identified the causative organism as *Cercaria longicauda* and suggested that the black teal was the definitive host. However Andrews (1976)[4] believes that this was a misidentification and that the host of the adult schistosome is the New Zealand scaup (*Aythya novaeseelandiae*). The intermediate hosts are pulmonate snails of the genera *Myxas* and *Limnaea*. Schistosome dermatitis has been reported from several lakes in New Zealand including Wanaka, Hayes, and Wakatipu and is more of a nuisance than a serious medical problem.

Conclusions

There is a dearth of information on all parasitic zoonoses in New Zealand with the exception of hydatid disease. More research is needed on the prevalence of these parasites in both the human and animal hosts before the risk to humans can be properly assessed. It seems probable that at the present time some cases of parasitic zoonoses go undiagnosed. The possibility that other zoonoses such as those caused by *Fasciola hepatica* Linnaeus 1758 and *Sarcocystis lindemanni* (Rivolta 1878) occur cannot be excluded. With the large increase in travel the possibility of the introduction of new diseases exists especially from South East Asia and the Pacific region. However the climate of New Zealand and then the lack of certain important disease vectors, such as Anopheline mosquitoes, reduces the possibility of local transmission. Possibly the cool or freezing climatic conditions found in New Zealand at certain times of the year account for the absence of *Angiostrongylus cantonensis* (Chen 1935) in the rat population (Alicanta and McCarthy 1964).[2] This parasite is known to cause meningoencephalitis in humans in the Pacific, South East Asia and Australia. One zoonotic disease which could become endemic in parts of the country is cutaneous larval migrans caused by the dog hookworm (*Ancylostoma caninum* (Ercolani 1859) and *Ancylostoma braziliense* (Gomez de Faira 1910)). *A. caninum* has been imported into New Zealand from Australia in dogs (Smith and Hooke 1975)[23] and a human case of cutaneous larval migrans contracted in Florida, U.S.A. was treated in Christchurch (Muir 1966).[20] Clinicians should be aware of the possibility of seeing patients with zoonotic diseases and technologists should be fully acquainted with the diagnostic techniques so that appropriate treatment can be commenced as rapidly as possible.

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Campylobacter Septicaemia
A Case Study

Mrs Anne Paterson
Dunedin Hospital

Received for publication December 1980

Introduction and Summary

There are two campylobacter species that cause human infection; *Campylobacter fetus ss jejuni* which causes acute gastro-intestinal infection and is proving to be a common identifiable cause of infectious diarrhoea, and *Campylobacter fetus ss intestinalis* which is important as a cause of human systemic disease and is the campylobacter species most frequently associated with septicaemia. The very young and old or the immunologically impaired are the most susceptible to serious infection with *C. fetus ss intestinalis.*

This paper reports a case study of septicaemia caused by *Campylobacter fetus ss intestinalis* and an attempt is made to explain how an elderly gentleman with varicose veins acquired this infection.

Case History

Mr E. is an elderly gentleman who has had varicose veins of the legs, for the past 40 years, with recurring varicose ulcers in the latter years. He had previously received successful sclerotherapy for his varicose veins. (Sclerotherapy is a non-surgical treatment for superficial varicose veins, which involves the injection of a chemical irritant, such as ethanolamine oleate, into the vein. This causes phlebitis of the vein and effectively "shuts off" the affected vein from the blood circulation.) Mr E. had had no complications with his sclerotherapy in previous years and was scheduled for further sclerotherapy early in 1980 after a varicose ulcer on his left ankle had healed satisfactorily. Mr E.'s general practitioner administered sclerotherapy on Friday, 25 January. Over the weekend Mr E. had an acute but self-limiting episode of mild diarrhoea, which had completely resolved by the following Friday, 1 February, when he returned to his doctor for further sclerotherapy. Within 24 hours of the second injection Mr E. had developed an ileofemoral thrombosis with oedema from below the umbilicus, involving the lower abdomen, genitalia and left thigh. Consequently he returned to his G.P. who initiated daily intramuscular penicillin therapy. Mr E. had no further complications until 15 February when he developed cellulitis of the left thigh and became feverish and confused. On admission to hospital the following day he was noted to be toxic, mildly jaundiced, have a left ileofemoral thrombosis, cellulitis of the left leg and mitral incompetence which was already under cardiology surveillance. A diagnosis of probable septicaemia was made. In view of the prior sclerotherapy, the initial concern was for staphylococcal, or streptococcal septicaemia which, particularly with the mitral valve disease already present, had the potential to develop into endocarditis if incorrectly or insufficiently treated. Staphylococcus is the most common pathogen involved in septic complications following sclerotherapy (personal communication, Dr R. Meech).

On the evening of admission, six sets of blood cultures were taken at spaced intervals. Each set comprised a 100 ml thioglycollate broth and 100 ml liquoid broth to which were added 5-10 ml blood. The laboratory was not notified of the G.P.'s penicillin therapy until 36 hours after the blood cultures had been collected so β lactamase (to neutralise the penicillin) was never added to any of these. No further blood cultures were taken until well into convalescence.

Routinely blood cultures are incubated 48 hours at 37°C during which time they are watched for signs of growth (RBC lysis, turbidity, gas production). After 48 hours' incubation, all blood cultures are gram stained and subcultured onto a 5% sheep blood agar plate—incubated anaerobically for 72 hours at 37°C and a 10% heated sheep blood agar plate—incubated for 48 hours at 37°C in 10% CO_2. At their first subculture after 48 hours' incubation the blood cultures did not show any signs of growth. Direct gram stain at that time was noted as "no organisms seen" and there was no growth aerobically after 48 hours in 10% CO_2 at 37°C. When the anaerobic jars were opened after 72 hours, seven of the twelve bottle subcultures, showed a very light to a moderate growth of small translucent grey colonies, approximately 1 mm in diameter. Of the six sets of blood cultures, at least one bottle of each set showed growth. Four growths were via the thioglycollate broths and three via the liquoid broths. Gram staining of these colonies revealed fine gram negative bacilli which ranged in shape from slightly curved to "S" shaped and on occasion short spirals. On rechecking the Gram stains of the original (broth) cultures made at first subculture, these were seen to contain scanty numbers of similar but very faintly stained organisms. The distinct morphology of the organisms in the Gram stain combined with growth only via the anaerobic medium suggested a Campylobacter species. This was proved by biochemical testing and the organism fully identified as *Campylobacter fetus subspecies intestinalis.* (Technical Communications N.H.I. see Table I.)

Serological tests were performed to confirm the diagnosis of septicaemia by *C. fetuss ss intestinalis;* and to disprove earlier suspicions of staphylococcal or

Table I

	C. fetus ss intestinalis	C. fetus ss veneralis	C. fetus ss jejuni (C. coli)
Growth at 25°C	+	+	−
37°C	+	+	+
42°C	−	−	+
H_2S on TSI	−	−	−
H_2S lead acetate strips	+	−	+
Catalase	+	+	+
Oxidase	+	+	+
Growth 1% glycine	+	−	+
Motility	+ rapid & darting		+

streptococcal involvement. Only convalescent sera were available for testing. The Anti streptolysin O Titre and Anti DNAse-B levels were normal and static on successive testing. A.S.O.T. was performed by the Standard Microtitre Technique. Anti DNAse B was performed by Wampoles Tube Test Kitset. The Anti Staphylolysin Titre was negative by Behringwerke Kitset.

The serum was tested for antibodies to the *C. fetus ss intestinalis* isolate by the Standard Agglutination Technique using whole cell, heat killed antigen. The titre approximately two weeks following the septicaemia was 1:1024. Six months later, at the time of writing it had dropped over successive testing to 1:512.

Sensitivity testing carried out anaerobically by the Kirby-Bauer disc technique showed the organism to be: penicillin resistant, erythromycin sensitive, doxycycline sensitive, co-trimoxazole sensitive.

The antibiotic therapy on hospitalisation was very high doses of penicillin and cloxacillin (N.B. two weeks penicillin prior to hospitalisation). It was changed to cephradine when a penicillin hypersensitivity rash appeared. When the culture and sensitivity results became available, the therapy was amended to erythromycin.

Conclusions

Thus how did Mr E., who originally had only varicose vein problems acquire a campylobacter septicaemia?

After recovery from the septicaemia Mr E. was shown to have a histamine fast achlorhydria related to parietal cell antibodies. Thus an initial defence system is missing which enables smaller inocula of any potential pathogen to enter the gastrointestinal tract. Although faeces were cultured for pathogens during convalescence, Campylobacter species were not looked for. So it appears that Mr E. who had no contact with any of the reported sources of campylobacter such as raw poultry, pets and unpasteurised milk (1, 7, 8, 9) somehow ingested the *C. fetus ss intestinalis.* This organism though an unusual cause of enteritis, probably caused the mild attack of diarrhoea which occurred following the first sclerotherapy. The organism was still resident in the large intestine when the ileofemoral thrombosis developed as a complication of the second sclerotherapy. Oedema around the rectum increased the permeability of the gut wall to all potential pathogens present and it was the *C. fetus ss intestinalis* which was most virulent and invaded the blood stream[3, 4].

The *Campylobacter fetus* subspecies to most commonly cause diarrhoea is *Campylobacter fetus ss jejuni*[2, 5, 8, 9, 10]. The National Health Institute reports widespread isolation of this thermophilic campylobacter from predominantly small children and young persons with diarrhoea[5].

In contrast the subspecies intestinalis has only been reported twice previously in the last four years to the National Health Institute, both in 1978—case in which it has caused serious illness in compromised hosts such as diabetics and alcoholics.

Summary

This case demonstrates the need to be aware of all Campylobacter species, not just the diarrhoea causing thermophilic campylobacter. As medical technology increases the number of compromised hosts, so we must become more aware of further opportunistic pathogens such as *Campylobacter fetus subspecies intestinalis.*

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The Separation of Serum IgM by Gel-filtration for use in Toxoplasma Indirect Immunofluorescence

Ray T. M. Cursons and Sherryn Cepulis
Department of Pathology, Waikato Hospital, Hamilton

Received for publication October 1980

Summary

Gel filtration using Bio-Gel A-5 was found to be a simple efficient method for the separation of serum IgM from IgG. The use of the IgM fractions in the indirect fluorescent antibody method avoided the reporting of false negative results in 7 out of 31 serum samples examined.

Introduction

Toxoplasma gondii, the aetiological agent of toxoplasmosis in man and animals, must be considered one of the most prevalent zoonotic diseases of man. In the United States of America, it is estimated that in excess of 50% of the population have antibodies to *T. gondii.* Krick and Remington (1979)[3]. In general, infection of adults with *T. gondii* is asymptomatic, with the most common clinical manifestation observed being lymphadenopathy. Although the isolation of *T. gondii* from body fluids or biopsy material via intraperitoneal passage in mice is sometimes practised, the primary method of diagnosing toxoplasmosis is using serology.

The classical serological diagnosis of an infectious agent depends on the demonstration of an increase in specific antibody titres between paired, acute and convalescent sera, or alternatively, the observations of a dramatically elevated specific titre, particularly IgM. The advantage to the clinician of using IgM titres is that acute infections can be diagnosed, and so save time lost by waiting for the results of convalescent sera. However the use of the IgM indirect fluorescent antibody (IFA) test is complicated in that unless IgM is separated from IgG, false negatives due to competition between these two immunoglobulin classes for the same antigenic binding sites can arise; Cohen *et al.* (1967)[2], Pyndiah *et al.* (1979)[4]. Furthermore, false positives can also arise if antinuclear antibodies and rheumatoid factors are also present; Pyndiah *et al.* (1979[4], Caruana (1980)[1]. Traditionally the separation of IgM from IgG involves sucrose density ultracentrifugation. Recently Pyndiah *et al.* (1979)[4] have described a simple, efficient, inexpensive alternative method of separating IgM from IgG using gel-filtration on Bio-Gel A-5 m.

This paper describes our experience with this technique and its application to toxoplasma immunofluorescence.

Materials and Methods

Serum Samples

Patient's sera submitted to the Department of Immunology for anti-toxoplasma titres were used in the study. These samples were stored at −20°C before being tested for toxoplasma antibodies by both indirect haemagglutination (IHA) and IFA. Those sera having an anti-toxoplasma titre in excess of 1:512 or when particularly asked for by the clinician were then both analysed by the IgM-IFA using gel-filtration separated—and unseparated IgM.

Gel-filtration of Serum

This method was similar to that originally used by Pyndiah *et al.*[4] except for two modifications. A Pharmacia chromatography column (1.2 cm

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internal diameter) was used instead of the 5.0 ml polypropylene concentric disposable syringe and a Buchler peristaltic pump to achieve a constant flow rate. Briefly Bio-Gel A-5 m was poured into the column to a height of 4.0 cm. The column was then equilibrated with 0.5 M tris (hydroxymethyl) aminomethane-hydrochloride buffer, 0.15 M NaCl, 0.03% NaAz pH 7.2. The flow-rate was standardised at six drops/minute. 100μl of the sample serum was thoroughly mixed with 5μl of Dextran blue 2000 (Sigma) (50 mg/ml made up in the above Tris-buffer) and carefully applied to the top of the gel-bed using a pasteur pipette. The void volume (about 1.5 ml) was discarded and the Dextran blue fraction (about eight drops) was collected after elution from the column. Thereafter five consecutive two-drop fractions were collected followed by three consecutive four-drop fractions. The column was then rinsed with 5.0 ml of Tris buffer and the next sample applied. All fractions were then tested for the presence of IgG and IgM using anti-human immunoglobulin (Oxford) by countercurrent immunoelectrophoresis at pH 8.6.

Countercurrent Immunoelectrophoresis (CIE)

1.0 g of Pharmacia Type B agarose was dissolved in 100 ml of pH. 8.6 Barbiturate buffer (ionic strength 0.5) by boiling for 10 minutes. When cooled to about 50°C, 3.0 ml of this solution was poured onto a standard microscope slide (76 × 26 mm). After solidification 2.5 mm circular wells were cut at a distance of 2 mm apart. Oxford antihuman serum was placed in the anode wells and serum fractions in the cathode wells and subjected to electrophoresis at a constant current of 5 m A/slide for 30 minutes. Thereafter the slides were left at 4°C for one hour washed in saline for six hours, dehydrated and then stained with Naphthalene black to observe any precipitin lines.

Indirect Haemagglutination Test

This was performed as set out by the manufacturer (International Biological Laboratories).

Indirect Fluorescent Antibody Test

Serum samples, diluted in 0.05 M pH 7.2 phosphate buffered saline (PBS), were placed on commercially prepared Toxoplasma-antigen-slides (Microbiological Research Group) and incubated at 37°C for 30 minutes. The slides were then washed twice in PBS for 10 minutes and stained for 30 minutes at 37°C with either a 1/40 dilution of fluorescein isothiocyanate (FITC) labelled anti-human serum (Hoechst) or a 1/20 dilution of FITC labelled anti-human IgM (Kallestadt), using Evans blue (0.2% w/v) as a counterstain. Thereafter the slides were rinsed twice again in PBS, rinsed once with distilled water, mounted in pH 9.0 glycerol-carbonate-buffer and examined under 400 × magnification using a Zeiss incident microscope (exciting filter 450-490 nm, beam filter 510 nm, barrier filter 520 nm) equipped with a 100 Watt quartz halide lamp.

Results

(a) Use of Bio-Gel A-5 Gel-filtration in the separation of serum IgM from IgG

The methodology of Pyndiah *et al.* (1979)[4] was initially investigated. However their methodology was found to give inconsistent results with respect to the resolution and distribution of the immunoglobulin classes eluted from the column. By using a peristaltic pump to obtain a constant flow rate and a commercial column to minimise mixing in the dead space at the outlet both the resolution and consistency of operation were vastly improved. As shown in Table I, IgM (because of its greater M.W.) was eluted first with the Dextran blue. The Dextran blue thus served both to monitor the homogeneity of the column and to act as a marker for the IgM fractions. In general the dilution factor of serum IgM resulting from the use of the column was found to be approximately 1/10.

Table I
Distribution of IgM and IgG in Bio-Gel A-5 m fractions

Fraction Number	IgM as detected by CIE	IgG as detected by CIE	Colour
1 (8 drops)	+/−	−	Blue
2 (2 drops)	+	−	Blue
3 (2 drops)	+	−	Blue
4 (2 drops)	+	±	Bluish
5 (2 drops)	+/−	+	Clear
6 (2 drops)	−	+	Clear
7 (4 drops)	−	+	Clear
8 (4 drops)	−	+	Clear
9 (4 drops)	−	+	Clear

(b) Serological Titres to Toxoplasma

Table II shows the serological titres for the 31 sera obtained using IHA, IFA, and IgM-IFA techniques. The variation in titres observed be-

Table II
Serological Titres to Toxoplasma

Patient Number	Indirect Haemagglutination Titre	Indirect Fluorescent Antibody Titre	IgM-Indirect Fluorescent Antibody Titre	Column IgM Indirect Fluorescent Antibody Titre
1	1:1024	1:256	<1/8	—
2	1:256	1:64	1/16	1/10
3	1:256	1:128	<1/8	—
4	1:128	1:16	<1/8	—
5	1:16,384	1:1024	1/16	1/10
6	1:512	1:1024	1/64	1/40
7	1:2048	1:1024	1/16	1/10
8	1:512	1:1024	1/128	1/80
9	1:512	1:128	<1/8	—
10	1:128	1:128	<1/8	—
11	1:512	1:512	<1/8	1/20
12	1:1024	1:2048	<1/8	1/20
13	1:512	1:64	<1/8	—
14	1:512	1:128	1/8	1/20
15	1:1024	1:256	<1/8	—
16	1:1024	1:1024	<1/8	1/20
17	1:512	1:512	<1/8	—
18	1:1024	1:256	<1/8	—
19	1:2048	1:512	<1/8	1/10
20	1:2048	1:1024	<1/8	1/20
21	1:1024	1:512	<1/8	—
22	1:4096	1:512	<1/8	—
23	1:512	1:256	1/32	1/40
24	1:1024	1:256	<1/8	—
25	1:1024	1:256	<1/8	—
26	1:1024	1:256	<1/8	—
27	1:512	1:128	<1/8	1/40
28	1:512	1:256	1/16	1/20
29	1:256	1:256	<1/8	—
30	1:1024	1:512	<1/8	—
31	1:2048	1:512	<1/8	1/10

Table III
Comparison between IgM Positive-IHA Titres and-IFA Titres

Titres	Indirect Haemagglutination Titres also IgM Positive	Indirect Fluorescent Antibody also IgM Positive
>1:2048	5/6	1/1
1:1024	2/10	5/5
1:512	7/10	3/7
1:256	1/3	2/9
1:128	0/2	2/5
1:64	0/0	1/2
1:16	0/0	0/1

tween the IHA and IFA results is as expected, since the former technique measures antibodies directed against soluble cytoplasmic antigens which appear later in the course of the disease as compared with the IFA surface-antigen-directed antibodies. The IgM-IFA results demonstrated the necessity of using fractionated sera to prohibit antibody competition in that 7/31 sera exhibited false negatives when stained using unfractionated sera. Table III shows the breakdown of IHA and IFA results in relation to a positive IgM result. Only IFA titres of 1:1024 or greater, correlated with a positive IgM titre. Of particular interest was serum number 2 which had a positive IgM but low IHA and IFA titres. On investigation this

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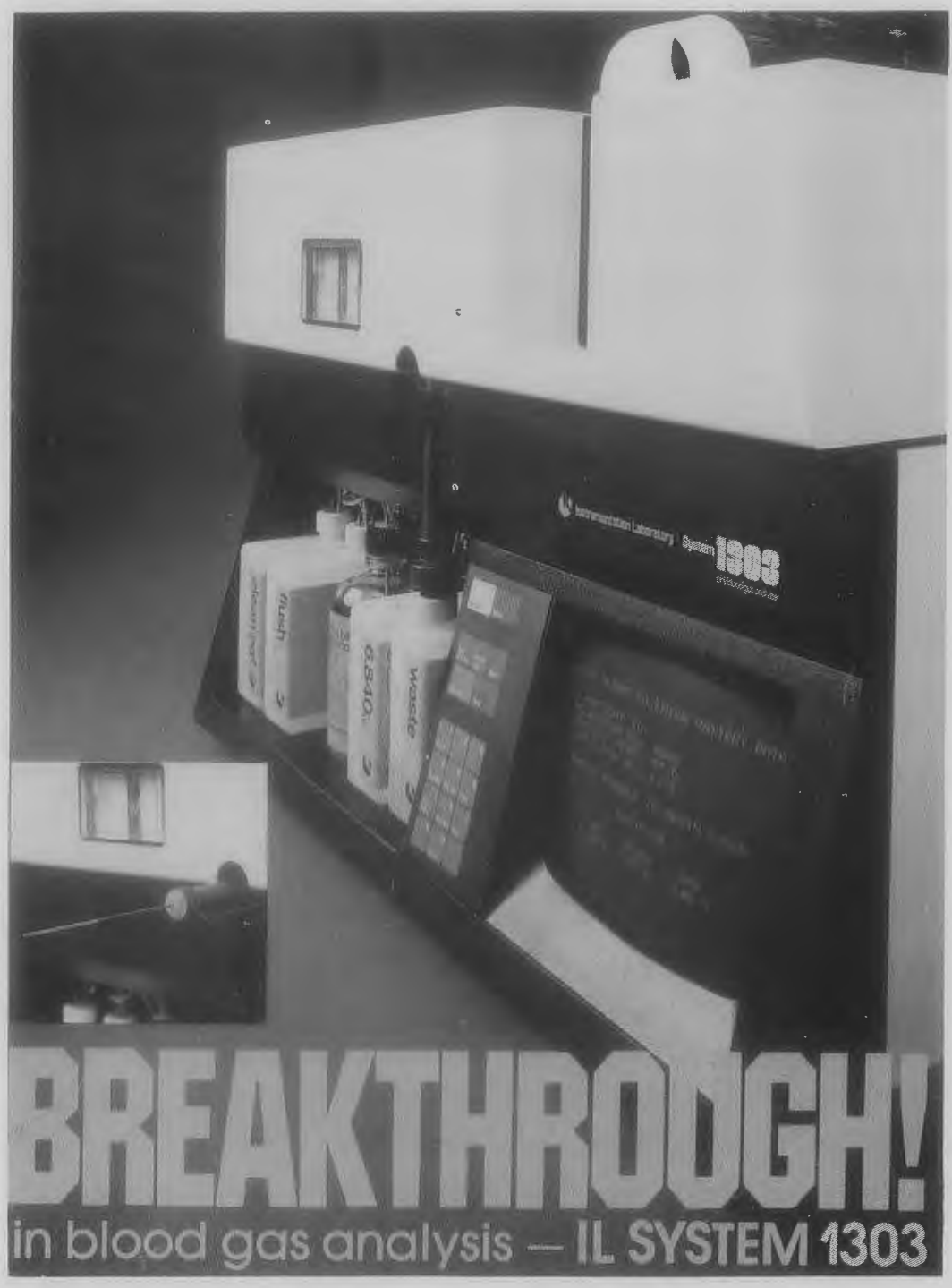

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813

serum was shown to be from a positive case of toxoplasmosis known to be at least six months old. No false positives (i.e. positive serum IgM but negative fractionated IgM) were observed.

Discussion

The results obtained demonstrate that serum IgM can be efficiently separated from serum IgG via simple column chromatography using Bio-Gel A-5m as previously found by Pyndiah *et al.*[4] The relative inexpense and small volume of serum required for the procedure should make it a viable alternative in those smaller laboratories which do not possess an ultracentrifuge, and thus help to reduce the possibility of false negative results where paired sera are not available.

Furthermore, providing the dilution factor is not too great, the procedure could also be used in the diagnosis of virus specific IgM, as originally suggested by Pyndiah *et al.* (1979)[4].

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A New Eosinophil Diluting Fluid

J. E. Lucas, A.N.Z.I.M.L.T.
Haematology Department, Dunedin Hospital

Received for publication December 1980

Summary

An aqueous solution of the indicator Bromchlorphenol blue is used in place of the traditional acidic dyes, e.g., eosin in Dunger's solution, as a stain for obtaining absolute eosinophil counts in a counting chamber.

Introduction

It has been known, since Ehrlich (1879)[2] first described the eosinophil in blood, that the specific granules take up certain acidic dyes. Much more is now known about eosinophils including the fact that the specific granules contain the basic protein arginine and it is this substance for which the acidic dyes show an affinity. Gleich *et al.* (1973)[3]. This fact has been made use of over the years to produce stains which enable the eosinophils to be selectively stained and counted in a counting chamber. This method provides a more accurate total eosinophil count than that provided by calculation with the total leucocyte count and percentage eosinophil count in the 100 cell differential due to inherent problems of accuracy in this procedure. Barnett, C. W. (1933)[1]. The principles of this staining technique have been reviewed by Speirs (1952)[8].

Dunger's fluid contains three main components: (a) an acidic dye (e.g., eosin); (b) water to lyse the erythrocytes; and (c) 5-10% acetone which inhibits the lytic action of water on leucocyte membranes. One modification utilises propylene glycol to lyse the erythrocytes and sodium bicarbonate to lyse all leucocytes except eosinophils. Pilot, M. L. (1950)[6]. With all the staining fluids, blood is diluted to 1:10 or 1:20 and placed in a Fuchs-Rosenthal counting chamber. For eosinophils to be optimally visualised, a very bright light is required which is unpleasant and tiring to work with.

Bromchlorphenol blue (dibrom-dichlorphenol-sulfonphthalein) is an indicator dye which has a pH range of 2.8-4.6 with a corresponding colour change of yellow to purple. This substance has recently been utilised in staining iron deposits in erythroblasts, in itself a fortuitous discovery, as was the statement in the original article that eosinophils were readily visible on the marrow films. Kass *et al.* (1978, 1979)[4, 5]. That finding was confirmed in this laboratory and an attempt was made to allow this phenomenon to be utilised in "wet-chamber" counting of eosinophils.

A 0.1% aqueous solution was used and it was found that eosinophils stained readily and strongly. Counts with this fluid compared favourably with those obtained with our standard diluent at that time (Randolph's fluid), as did

counts prepared from figures obtained by calculation from the differential count, suggesting that the dye was selectively staining eosinophils. Randolph (1944)[7]. The outstanding feature is the extreme ease with which eosinophils can be recognised. They are seen as clusters of dark purple granules around or obscuring the translucent nucleus. Other leucocytes are seen as pale unstained bodies. A high degree of illumination is not required.

Materials and Methods

Reagents

*0.1% Dibrom-dichlorphenol blue[a] in distilled water. Filter and store in the refrigerator at 4°C.

*Bromophenol blue[b] may be substituted.

a Matheson, Coleman and Bell. Norwood, Ohio, USA.

b May and Baker Ltd. Dagenham, England.

Note: Acetone is not added to this solution to inhibit lysis of the leucotyes. The addition of acetone causes a precipitate.

The solution is stable indefinitely at 4°C.

Dilute whole blood 1:10 or 1:20; flood a Fuchs-Rosenthal counting chamber and allow cells to stain and settle for five minutes in a wet chamber. Counts may be performed at any convenient time after this period as counts appear to be stable for up to six hours without any noticeable lysis of the eosinophils.

Discussion

Bromchlorphenol blue is an excellent substitute for the historic eosinophil count diluents. It renders the eosinophils clearly visible and has added advantages in being easy to prepare and indefinitely stable at 4°C.

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Blood Glucose Monitoring: Dextrostix and Boehringer Test Strips Compared in a Semi-Quantitative and Quantitative Role

R. McKenzie, A.N.Z.I.M.L.T.
Biochemistry Department, Nelson Hospital, Nelson

Received for publication January 1981

Summary

Four methods of performing blood glucose levels were tested. Dextrostix and BM 20-800 strips were compared with the appropriate colour chart. The reagent strips were then used in conjunction with a reflectance meter. Dextrostix were used with the Ames Eyetone meter and the Boehringer Reflomat was used with the Reflotest strips. Dextrostix were at best semi-quantitative though they performed well in the hypoglycaemic range. BM 20-800 strips were consistent but read low. Both meter systems performed well with the Reflotest system being less subject to errors. Comprehensive patient instruction on operation and maintenance of meters is desirable to obtain best results.

Introduction

The object of the study was to compare the performance of the test strips rather than the meters. Dextrostix used in conjunction with the other meters available (Hypocount, Glucochek) can give similar results to the Eyetone and are subject to similar variations, Webb *et al.* (1980)[1]; Webb *et al.* (1972)[2], The Reflamat is the only instrument available for use with the Reflotest strips at the present time.

Methods

Blood samples for initial comparison were obtained from finger prick samples collected in capillary tubes. BM 20-800 (Boehringer Mannheim, New Zealand Agents Smith-Biolab Ltd) and Dextrostix (Miles

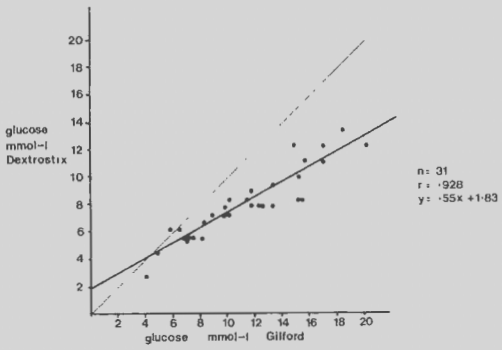


Figure 1.

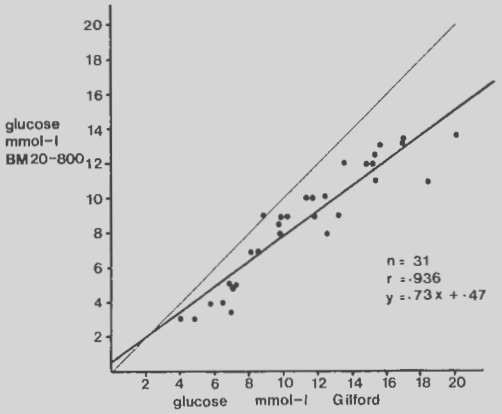


Figure 2.

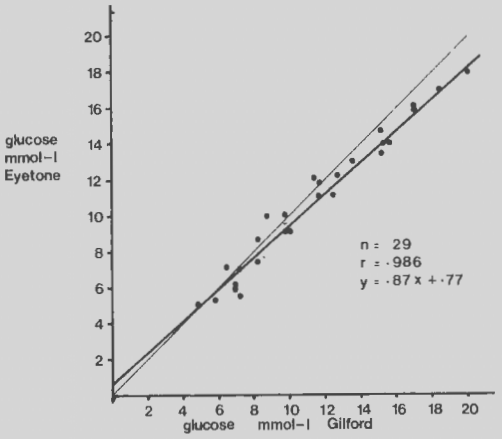


Figure 3.

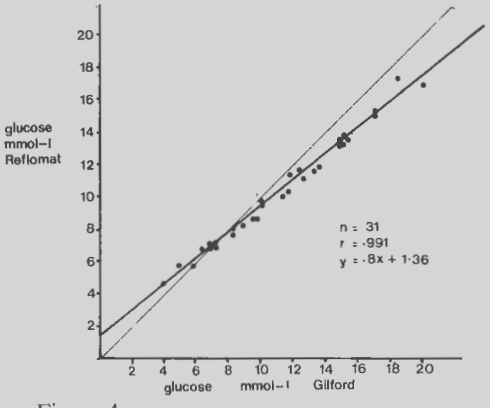


Figure 4.

Laboratories, New Zealand Agents Ebos Dental and Surgical Supplies) estimations were performed, the samples were then assayed with the meters. A capillary sample was also centrifuged and assayed using the laboratory's routine glucose methodology. The laboratory's routine glucose method is a glucose oxidase/peroxidase method with 4-amino-phenazone as the chromogenic oxygen acceptor, performed on a Gilford 3500 discrete analyser. The BM 20-800 strip for matching with a colour chart has two colour patches on each strip. This is designed to make matching easier and to increase sensitivity over the whole range of glucose values, one patch has marked colour steps in the lower range, the other in the higher range. The Reflotest strip for use with the Reflomat has a single colour patch. The Dextrostix used in the meter are the same as the ones for visual comparison. Precision studies were performed on both instrument systems. The sample used was heparinized venous blood and these precision runs were performed in both the normal and elevated glucose range. Standard deviations in coefficient of variations were calculated. The next four methods were next evaluated in the low range. Heparinized venous samples were taken at 0900 hours from three people with normal glucose levels. A glucose estimation was performed using all four systems and an aliquot centrifuged for assay by the Gilford 3500. Samples were then incubated at 37°C for three hours to allow glycolysis to proceed and the exercise repeated. It was repeated after a further three hours then another two.

The Dextrostix method requires that blood samples be left on the strip for one minute, washed off, blotted and the colour compared either by chart or meter. The Reflotest and BM 20-800 methods involve wiping the drop of blood from the test strip with a cotton wool swab after one minute and comparing the colour either with a Reflomat or colour chart at two minutes. Values above 13.3 mmol/l are compared at three minutes with BM 20-800 strips.

Experiments were done with Dextrostix, varying the time when the blood was washed off. This time variation extended 30 seconds either side of the desired

60 second interval and was performed on both normal and raised glucose values and measured with the Eyetone. A similar exercise was performed with the Reflotest strips varying the wiping time before reading with the Reflomat. The wiping time was also kept constant in one experiment with the Reflotest/Reflomat system and the time before insertion in the meter varied. These results were also recorded in the normal and elevated range. The final stage involved getting two untrained persons to repeat part of the exercise. Neither had previous experience of glucose test strips or meters. They were given brief instruction on the use of the instruments, had a practice run, then performed a normal and elevated glucose assay using all four systems. Each level was done three times with the meters to give an idea of reproducibility.

Results

i. Results obtained in comparing 31 samples (29 Eyetone) with results from Gilford 3500 are shown in Figures 1-4.
ii. Results of precision studies—see Table I.

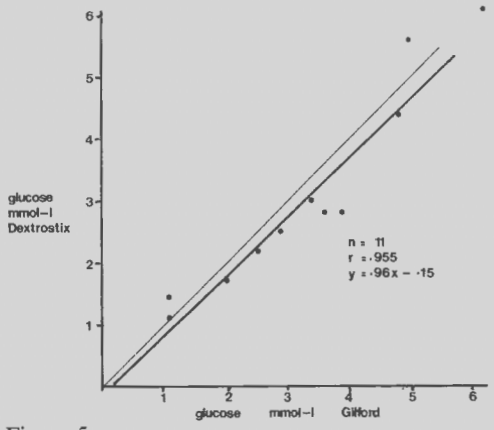
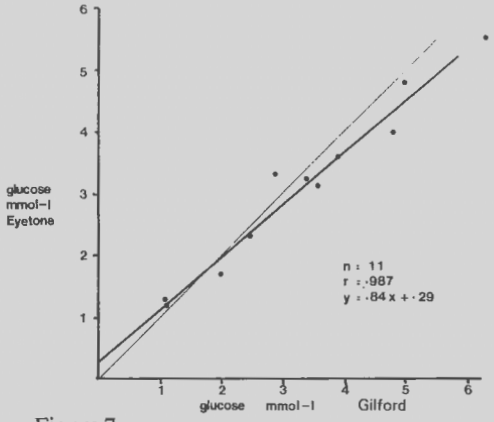


Figure 5.



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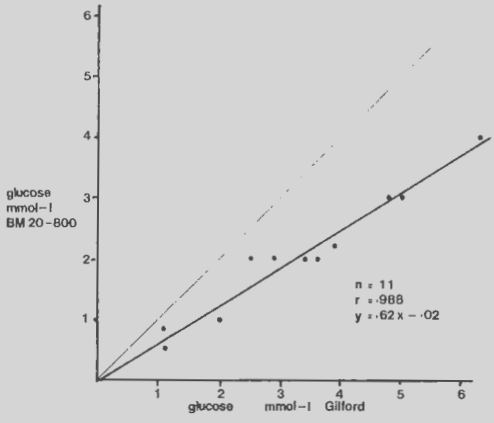

Figure 6.

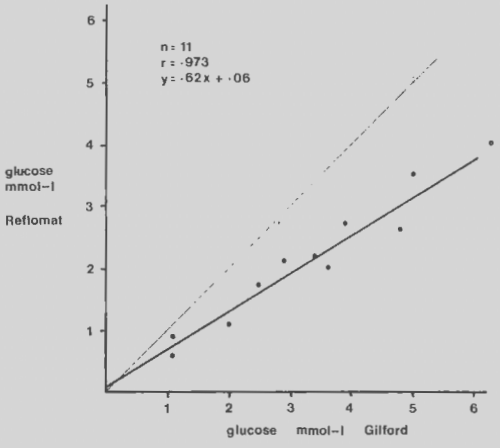

Figure 8.

Table I

Eyetone	(low level)	n = 10	$\bar{X}$ = 5.3	SD 0.4	CV 6.8%
Eyetone	(elev. level)	n = 10	$\bar{X}$ = 13.1	SD 0.6	CV 4.4%
Reflomat	(low level)	n = 10	$\bar{X}$ = 5.9	SD 0.1	CV 1.8%
Reflomat	(elev. level)	n = 10	$\bar{X}$ = 13.6	SD 0.4	CV 3.2%

Table II
Normal glucose level. Variation of blood glucose level with time before wiping/washing strip.

(Seconds)	30	40	50	60	70	80	90
Reflomat (glucose mmol/l)	4.5	4.5	4.6	4.8	5.0	5.0	5.1
Eyetone (glucose mmol/l)	3.5	4.5	4.9	5.0	6.0	6.0	6.6

Table III
Elevated glucose level. Variation of blood glucose level with time before wiping/washing strip.

Seconds)	30	40	50	60	70	80	90
Reflomat (glucose mmol/l)	10.4	11.5	12.4	13.2	13.0	13.8	14.0
Eyetone (glucose mmol/l)	7.1	9.0	10.6	12.9	14.1	18.1	19.5

ii. Evaluation in low range
In evaluating the low range the BM 20-800 strips, Dextrostix and Eyetone were used as before. The Reflotest hypoglycemic strip was used in this range instead of the usual Reflotest strip. This has an alternative scale which attaches to the instrument and covers the range 0.55-8.5 mmol/l. The normal Reflotest strips cover the range 3.0-20 mmol/l. These results are shown in Figures 5-8.

iv. Results of varying washing/wiping times—see Tables II, III, IV.

v. Assays by untrained persons—see Table V.

Table IV
Variation of blood glucose levels with time after wiping strips and before reading (applicable to Reflomat only).

(Seconds)	40	50	60	70	80
Normal glucose level (mmol/l)	5.1	5.3	5.4	5.2	5.2
Elevated glucose level (mmol/l)	13.0	13.1	12.8	13.1	13.35

Table V

Operator 1	**Gilford**	**20-800**	**Dextrostix**	**Eyetone**	**Reflomat**
Normal range (mmol/l)	6.7	3.0	5.6	7.1 6.0 6.5	5.8 6.0 6.2
Elevated range (mmol/l)	16.1	12.0	13.9	19.0 20.5 21.5	15.8 15.9 15.8
Operator 2					
Normal range (mmol/l)	6.9	5.0	7.2	6.0 6.0 6.5	6.7 7.2 7.1
Elevated range (mmol/l)	16.1	13.0	13.9	21.0 19.0 18.5	16.8 15.2 15.5

Discussion

Comparing the Dextrostix with a colour chart was a difficult task in the middle and high range. Differences between the colour blocks at the high end of the scale is minimal and matching was mainly guesswork. The first four colour blocks have well defined steps and performance in this area was good. Performance in the high range was not satisfactory. Used with a colour chart Dextrostix can only give a very broad estimate of the true glucose level in the normal-higher range.

More consistent results were obtained with the BM 20-800 strips. Readings with the BM 20-800 strips were generally lower than results obtained with the meters. Other users also obtained lower results. It was not possible to obtain results matching those from a correctly operated reflectance meter with BM 20-800 strips. BM 20-800 strips were, however, easier to use than the Dextrostix and the results obtained were superior. Performance definitely improved with practice and with certain patients these could be sufficiently accurate to improve diabetic control.

Dextrostix used with the Eyetone gave results marginally better than the Reflomat system in the initial study. It must be realised that in this study the timing was strictly adhered to and the washing and wiping techniques were standardised as best was possible. Any deviation from this "ideal" situation and it is obvious the Reflotest strip is superior. The BM 20-800 and Reflotest strips had other advantages. It was easier to apply the drop of blood and get an even coverage with both of these strips compared with the Dextrostix. The surface of these strips are plastic and smaller than the Dextrostix. The Dextrostix with their rough absorbent surface were at times difficult to cover consistently. The BM 20-800 and Reflotest strips were also better packaged. They both are loose packed in alloy containers, 50 per container. The Reflotest kit contained new calibration strips for each batch and a control, these were both extra items with the Eyetone system.

A disadvantage with the Reflomat system was the need for a separate kit for the hypoglycemic range. One strip that covered the whole range would be preferable. Overall the Reflotest strips performed better than Dextrostix. The Reflotest/ Reflomat system is a relative late-comer to the New Zealand market, of the five meter systems at present available four utilise Dextrostix. The use of these instruments (Eyetone, Hypocount, Glucochek) is well established. It has been demonstrated that significant errors can occur with timing variations with Dextrostix. Other factors can cause errors also. Some of these factors are, washing strips too vigorously or not long enough, insufficient sample on strip, waiting too long after washing before inserting in instrument, blotting strip too long, old standards and faulty controls.

However it has also been demonstrated that good results can be obtained with the system.

A patient should be able to obtain similar results if instructed thoroughly in the use, maintenance and quality control of their instrument. This instruction is already taking place in some of the larger centres but is not in Nelson and possibly not in other localities.

Ideally when a patient purchases or borrows a reflectance meter they should bring it to the laboratory. Laboratory staff could explain the principles of operation of the instrument, the patient could practise and have samples checked by the laboratory and a suitable quality control and maintenance system could be devised. The effects of varying timing and washing of strips could be demonstrated to reinforce the importance of consistent technique. Patients who are being monitored with BM 20-800 strips could also practise reading these under supervision of laboratory staff and have their results checked. Patients owning meters have presented at our laboratory and have been surprised to learn that the amount of blood on the Dextrostix was critical and that timing was also. The untrained persons who used the Eyetone were both told the timing was important but did not fully appreciate this, consequently their results were high with the Eyetone.

Ames have introduced a new meter, the Glucometer, to the New Zealand market. The Glucometer uses Dextrostix and can be calibrated using low and high value calibration chips or a "wet" calibration can be performed by the operator using high and low standards and Dextrostix. The instrument is micro-processor controlled, can "remember" this "wet" calibration check even when switched off. A "wet" calibration is an advantage since it standardises the instrument with the individual operator and if the operator maintains a consistent style it can reduce some possible errors. The system is the only one totally independent of mains power and at $195.00 is cheaper than the other systems. Although this system is superior to the other systems using Dextrostix it still possesses the weaknesses inherent with Dextrostix.

A portable system similar to this new Ames meter but using the Reflotest strip system would be a a promising combination.

Acknowledgements
Thanks to Smith-Biolab for loan of Reflomat and supply of test strips.

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"Ralph" Glass Knives—their Effect on Paraffin and Resin Sections

Glenys Rothwell, A.N.Z.I.M.L.T.
Histopathology Department, Diagnostic Laboratories, Dunedin Hospital, Dunedin

Received for publication March 1981

Summary
The use of "Ralph" glass knives to obtain from tissue, embedded in paraffin or resin, sections showing greater cellular detail than can be obtained conventionally is described.

Introduction
In most histopathology laboratories there is the problem of cutting sections thin enough for cellular detail to be observed.

The "Ralph" glass knife may be used for this purpose and we observed its ability to cut thin sections of tissue embedded in four different embedding media.

Materials and Methods
"Ralph" knives
The "Ralph" knife is a long edged glass knife made from 25 or 38 mm wide glass. The knives are broken on the LKB knife-maker, Linder *et al.* (1978)[4]. They are used for cutting sections on the Sorvall JB4 microtome.

A razor blade holder was adapted by inserting longer screws so that these knives could be used on a Leitz rotary microtome. A knife holder is however available commercially.

Cutting
For cutting paraffin sections it was found to be easier if a floating out trough is attached to the knife. This trough was made from thick aluminium foil and attached to the knife with dental wax (Photo 1).

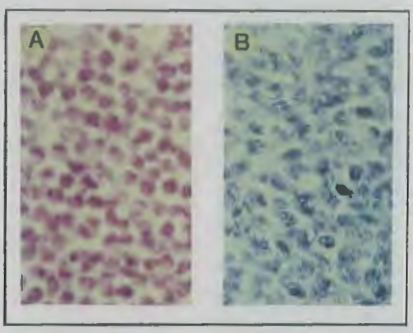
By varying the angle of the cutting edge it is possible to cut both resin blocks and paraffin blocks with these knives. The shallow angled knife is better for resin blocks while the steep angle is used for paraffin blocks (Photo 2). It was necessary to spray the cutting edge with a teflon spray. This prevented sections from adhering to the knife edge.

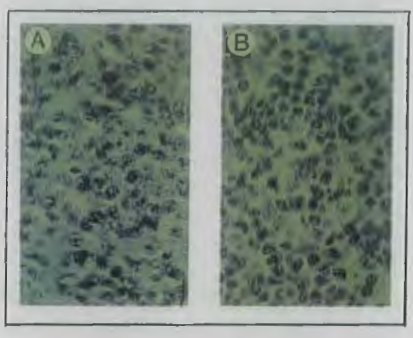
Embedding Media
Four different types of embedding media were used to observe the cutting ability of these knives. The media used were:
(a) "Mobil" paraffin wax—used routinely in our Histopathology Laboratory.
(b) Waterman's wax—a hard wax with a low melting point (Waterman 1939)[5].
(c) Epon—used routinely in electron microscopy.
(d) Glycol methacrylate (GMA)—an acrylic resin.

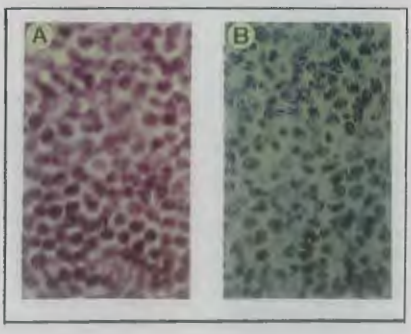
Method
Pieces of lymph node and kidney were fixed in 10 percent phosphate buffered formalin. Half of this tissue was post fixed in 2.5 percent glutaraldehyde in cacodylate (pH 7.5), in the hope that some of the finer structural detail would be preserved.

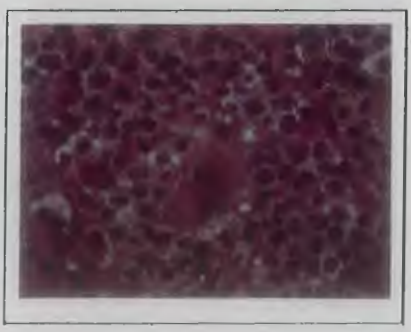
The fixed tissue was divided into four lots and both formalin fixed and post glutaraldehyde fixed tissue was processed to the four embedding media.

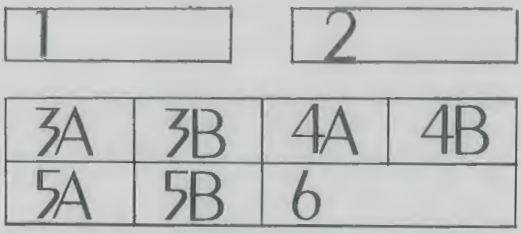
The tissues to be embedded in paraffin wax and Waterman's wax were processed by our routine processing schedules.











1. Razor blade holder adapted to hold "Ralph" glass knives, for use on Leitz rotary microtome.

2. "Ralph" glass knives showing shallow and steep angled cutting edges.

3. Lymph node—paraffin wax embedded, haematoxylin and eosin stain. Cut at 2 μm x 160.
(a) Cut with steel knife
(b) Cut with "Ralph" glass knife.

4. Lymph node—haematoxylin and eosin stain, cut with "Ralph" glass knife—2 μm thick x 160.
(a) Paraffin wax embedded
(b) Glycol methacrylate embedded.

5. Lymph node—haematoxylin and eosin stain. Cut at 2 μm x 160.
(a) Paraffin wax embedded, cut with steel knife
(b) Glycol methacrylate embedded. Cut with "Ralph" glass knife.

6. Reed-Sternberg cell in tissue embedded in glycol methacrylate. Haematoxylin and phloxine stain. Cut at 2 μm x 160.

That for Epon embedding was dehydrated in alcohol, before being placed in propylene oxide. From this it went into propylene oxide/Epon mixtures of increasing Epon concentrations before embedding in Epon.

The tissue for GMA embedding was dehydrated in alcohol and infiltrated twice over five hours in the infiltrating solution. We used a modification of Lee's method, Lee (1977)[3].

Infiltrating solution

2 Hydroxyethyl Methacrylate	91 ml (Analar BDH)
2 Butoxyethanol	9 ml (Analar BDH)
Benzoyl Peroxide	0.7 gm (Analar BDH)
Promotor Solution	
Polyethylene glycol 400	8 ml
N,N-dimethylanaline	1 ml (Analar BDH)

The infiltrating solution and promotor solution are mixed in the ratio of 30:1 to make the embedding solution.

The benzoyl peroxide acts as a catalyst in this reaction. Once the embedding solution is mixed there is little time before polymerization begins. However polymerization takes one to two hours before it is completed.

The Epon and GMA blocks were attached to wooden blocks with Five Minute Araldite before cutting on the Leitz rotary microtome.

Cutting

All sections were cut at 2 μm on the Leitz Rotary Microtome.

Sections of tissue embedded in paraffin wax, Waterman's wax and Epon were cut on to 30 percent alcohol in the trough. They were then floated on to distilled water at 54°C. The GMA sections were cut dry and then dropped from a height of 3-4″ on to cold distilled water.

The sections of tissue in paraffin wax and Waterman's wax were dried, dewaxed and taken to water, before being stained with Gill's haematoxylin and eosin, Gill *et al.* (1974)[2].

The GMA sections were dried thoroughly and stained with Gill's haematoxylin and counter-stained with 1 percent phloxine in absolute alcohol. Staining with eosin resulted in very feeble cytoplasmic staining. It is possible to remove the resin from these sections with careful controlled use of a resin solvent by pre-treating the sections with 5 percent sodium hydroxide in ethanol for two minutes, followed by rinsing in alcohol and water. It is then possible to use normal staining methods and times, Everiett (1979)[1].

The Epon sections were dried and stained with toluidine blue for half to one minute.

Results

See photographs 3, 4, 5 and 6. In each case "Ralph" knives produced greater nuclear detail.

Conclusion

"Ralph" knives were found to be suitable for cutting sections of all four embedding media. Of particular interest is their use for cutting thin 1-2 μm sections of lymph node, kidney and possibly bone marrow trephines embedded in GMA.

The cells of tissue embedded in GMA are not distorted and the nuclear detail is clearly defined unlike tissue embedded in paraffin wax.

It is possible to cut thin sections 1-2 μm thick, which are not easily obtained when cutting sections of tissue embedded in paraffin wax with steel knives.

Although GMA is expensive it has been found that the thin sections obtained aid in the diagnosis.

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Halophilic Vibrio Isolates from the Ear

Kay P. Bramley, Qualified Technical Assistant
B. M. Cornere, A.N.Z.I.M.L.T., Technical Bacteriologist
Department of Microbiology, Green Lane Hospital, Auckland

Received for publication April 1981

Summary

Halophilic vibrio infections were demonstrated in seven patients attending the Ear Nose and Throat Clinic at Green Lane Hospital between January 1975 and May 1980. Six isolates were identified as *Vibrio alginolyticus* and the other was confirmed as *Vibrio Heiberg group II.*

Introduction

Marine vibrios are well established as a cause of human gastroenteritis, Cawley (1972), and over the past 10 years much emphasis has been placed on their role in association with the ingestion of uncooked shellfish. More recently, halophilic vibrios have been incriminated in superficial soft tissue infections, Ryan (1976)[5], Pein, Lee and Higa (1977)[3].

This paper presents the results of routine cultures from all ear swabs to assess the frequency and relevance of halophilic vibrio species at this site.

Materials and Methods

Aural swabs were collected and routinely cultured on to the following media: sheep blood agar, MacConkey agar (with crystal violet) and Thiosulphate citrate bile salt sucrose (T.C.B.S.). Plates were incubated in 8-10 percent carbon dioxide with humidification at 37°C for 12-18 hours. The plates were then carefully examined. All gram negative bacilli were checked for their oxidase reaction, and further identified using API 20 Enterobacteriaceae (Analytab Products Incorporated) system and growth in 3 percent, 8 percent and 10 percent salt broth.

Organisms identified as vibrio species were confirmed by the National Health Institute, Wellington.

Results

After overnight incubation the vibrio species were seen as large, grey, moist spreading colonies on blood agar, non-lactose fermenting colonies on MacConkey agar and large yellow sucrose fermenting colonies on T.C.B.S. agar. They were oxidase positive, fermented specific carbohydrates and required salt broth for growth. Differentiating characteristics are given in Table 1. All *V. alginolyticus* strains required salt for growth, but *Vibrio Heiberg Group II,* grew in 0 percent and 3 percent salt broth. *Vibrio parahaemolyticus* failed to grow in 10 percent. The *Vibrio Heiberg Group II* was classified as a *V. cholera* using the API 20 E system and was subsequently confirmed serologically as a non-cholera *Vibrio Heiberg Group II* by the New Zealand Health Institute

In six cases the patients had a long history of chronic suppurative otitis media and previous surgery i.e. myringotomy and insertion of drain; myringoplasty (which in most cases failed due to unresolved infection) and one patient had a cholesteatoma removed on two occasions—see Table II. The infection had cleared after one week with the use of antibiotics in all cases and in three cases with aural toilet. All patients had a marked hearing loss, but this was most likely related to chronic infection and damage to the ear rather than the effect of this one organism. The following three case histories

Table I
Characteristics of Vibrio Strains

Test	V. cholera	V. alginolyticus	V. parahaemolyticus	Vibrio sp Heiberg Gp II
V.P.	−*	+	−*	+
Lysine	+	+	+	+
Arginine	−	−	−	−
Ornithine	+	−	+	+
Growth in 0% NaCl	+	−	−	+
3% NaCl	+	+	+	+
8% NaCl	−	+	+	−
10% NaCl	−	+	−	−

* The V.P. reaction for these organisms is variable.

Table II
Clinical Features of Vibrio Infections

Case	Age Sex (years)	Conditions	Treatment	Season		Associated Bacteria
1.	11 M	(R)	Soframycin (Topical) Ampicillin (Systemic)	Summer	1975 1980	*Staphylococcus aureus, E. coli, Acinetobacter, Candida, Alcaligenes faecalis*
2.	7M	(L)	Soframycin (Topical)	Summer		None
3.	19M	(L)	Aural toilet amoxil (Systemic) chloramphenicol (Topical)	Autumn		Yeast
4.	11M	Bilateral	Sofradex drops (Topical)	Autumn		None
5.	11M	(L)	Aural toilet	Summer		None
6.	7M	Bilateral	Soframycin (Topical) gentamicin drops chloramphenicol	Summer		*Staphylococcus aureus Haemophilus influenzae Streptococcus pyogenes*
7.	52M	Infected traumatic perforation	Aural toilet soframycin (Topical) chloramphenicol (Topical) amoxil (Systemic)	Summer		None

C.S.O.M. = Chronic suppurative otitis media.

demonstrate two examples of typical findings and one of unexplained cause.

Case 1: Child aged 7

This boy had a tonsillectomy in 1978 and ear discharge was noted then. In July 1979 he had a bilateral myringotomy and Teflon drainage tubes fitted into both ears.

During December and January 1979 the child spent a lot of time swimming in the sea and on his next visit to the E.N.T. Clinic in February 1980 a bloody yellow discharge was noted from his left ear. A swab was taken and *V. alginolyticus* was isolated. He was treated with soframycin and the infection cleared in one week.

Case 2: Child aged 11

This child had a history of recurrent otitis media. He was referred to the E.N.T. Clinic in August 1978 when a bilateral myringotomy was performed and Teflon drainage tubes were fitted in both ears. In December 1978 he went swimming in the sea and one week later developed a discharge. Mucus and pus were removed from his left ear by suction and *V. alginolyticus* was cultured. The ear was insufflated with antibiotics and definite hearing loss was noted in his left ear. By March 1979 the infection had cleared and he had a myringoplasty.

Case 3: Man aged 52

The patient was cleaning his ear with a cotton bud when he was jolted and perforated his ear drum. Throbbing and a bloody discharge followed. He was referred to the E.N.T. department and swabs grew *V. alginolyticus.* Aural toilet was performed and he was given soframycin and chloramphenicol drops. Oral amoxil was also administered. Two weeks later the ear was swabbed again and cultures were negative. One month later the perforation had completely healed and the ear drum was mobile. On further discussion the patient denied any contact with sea water before and after the injury so the source of the infection was not identified.

Discussion

These cases highlight the importance of the isolation of organisms usually associated with gastrointestinal conditions, Cornere and Cawley (1976)[2]. The halophilic vibrio species are common in coastal waters and while there were no specific geographical distribution or other features in common, the first six patients were young and swam frequently during the summer months. This seasonal variation coincides with the increase in sea water activity in the summer months, and is

also influenced by water temperature. It is possible that *V. alginolyticus* may be isolated at any time water temperatures are above 10°C. Rubin and Tilton (1975)[4].

The case of the 52-year-old male patient is interesting because he denied all contact with sea water immediately before or after his injury.

Five of the cases were school children, one was a linesman and one was a company director. All patients were males, and the organism was considered a secondary opportunistic invader. In any soft tissue infection occurring during the summer months associated with sea water the significance of *V. alginolyticus* should not be overlooked.

Acknowledgments

The authors would like to thank the Medical Superintendent and the Ear Nose and Throat Specialists, Green Lane Hospital, for permission to present this paper.

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Technical Communication

Gilford 3500—Reduction in Reagent Volumes With Emit[R] Techniques

Received 31 July 1980; Revised, 13 April 1981

A Gilford 3500 Chemistry Analyser has been used successfully in this laboratory for the past 24 months to assay samples for Theophylline using the Syva Emit technique. Bearing in mind calls for cost containment and the price of the reagents, we decided to attempt to reduce reagent volumes used per assay. Our modifications are cheap, simple, and do not adversely affect performance characteristics of the test.

The Gilford 3500 utilises three 2.50 ml syringes (dispensers A, B, and C) and a 100 µl syringe to pick up and deliver reagents and sample. Adjustment of reagent and sample volumes is accomplished by placing ¼" dia. stainless steel rods of various lengths, beneath the plungers, thereby varying plunger distance of travel. Reagents and sample are delivered into disposable plastic reaction vessels, which are placed on a moving series of racks. After a short pre-incubation period at room temperature, the contents of each vessel is aspirated into an electronically temperature controlled cuvette set to 30°C. The rate of change in absorbance is measured for 40 seconds. Zero calibrators are measured (in duplicate) first, an average reading calculated, and then subtracted from subsequent readings taken for the other calibrators, controls, and unknowns. Results are printed out in "delta absorbance" (DA) units per minute. Calibrator values are transferred manually to a blank supplied graph sheet, plotted, and the controls and unknowns read off. Conversion to µmol/l from µg/ml (in which the graphs are calibrated) is accomplished by using a multiplication factor of 5.55. All Gilford 3500 manipulations are automatic, as the instrument follows a series of instructions given it via a magnetic card just prior to batch start.

Reagents are supplied in the Syva kit, Emit® Theophylline Cat. 6P019. All are reconstituted according to directions given in the package insert.[1] In addition "working" reagents A and B are prepared just prior to batch start, by diluting the "stock" A and B with supplied buffer, in a ratio of 1:6.

Our modifications are to the standard Gilford methodology[2] which requires 10µl of sample to be delivered along with 250µl of buffer, followed by sequential addition of 300µl each of "working" reagents A, then B. We have reduced the "working" reagent A and B volumes to 200µl of each.

To achieve this end we have had made suitable lengths of ¼" dia. stainless steel rod for dispensers B and C (which deliver reagents A and B respectively). Full plunger travel of each syringe

is 60.0 mm. With 2.50 ml syringes fitted, full plunger travel allows delivery of 2.50 ml of reagent. 200 µl is 8 percent of 2.50 ml, we therefore required rod lengths of 60.0 mm less 8 percent, i.e. 55.2 mm. To reduce priming wastage on these dispensers, the outlet tubing was shortened by approximately 60 mm. Shortening of these tubes must, however, still permit attachment to the sampling tower in position 1, without strain or kinking. Having noted also that a relatively large amount of reagent was needed to fill the tubing between the sampling tower and the cuvette, we exchanged the supplied tubing for a piece of discarded reagent syringe outlet tubing (which has a smaller internal diameter).

With modifications complete, some loss of sensitivity was expected. This however, proved to be minimal, with only a slight reduction in calibration curve slope (DA difference between calibrator 1 and calibrator 5, was reduced from 105-110 units/min prior to modification, to 85-90 units/min after modification). Linearity within the calibrators range has remained unchanged. Precision studies involved monitoring the level measured for the Syva Theophylline control for two months prior to modification, with the following results:

n = 32, x = 83.1 µmol/l, S.D. = 4.7, C.V. = 5.7%, compared to levels measured for the same control for two months following modifications which gave:

n = 24, x = 84.3 µmol/l, S.D. = 4.4, C.V. = 5.1%. The published value for the control was 83.3 µmol/l.

Regression analysis comparing the modified to the original method gave:

n = 21, correl. coeff. = 0.96, slope = 1.06, y intercept = −2.9 µmol/l with samples ranging from 20 µmol/l to 140 µmol/l.

The total number of assays obtained per kit, has been increased from 100 to approximately 150. Actual cost per test varies according to batch size, which in our case is very variable (6-15 patient samples per batch). At an early stage we found that the standard curve obtained for each kit was very stable indeed throughout that kit's life, we therefore did not consider it necessary to run duplicate assays.

To perform Theophylline assays on the Gilford 3500 using the modifications outlined, requires that the technologist be a little more critical of instrument operation; calibration, adjustment, and cleanliness, etc., but we have found it well worth the extra effort. A well looked after and understood instrument performs the other routine higher volume tests processed on it at a better level of efficiency. Through carrying out modifications such as those outlined, our staff have come to appreciate the operational characteristics of the instrument in greater detail. Quite apart from improving the cost effectiveness of Emit ® assays, this experiment has also opened the way to reduction of reagent volume in many other of the assays that the instrument performs.

D. M. Fallas ANZIMLT,
Laboratory,
Thames Hospital.

References

1. Syva EMIT ® Theophylline, instruction leaflet.
2. Gilford 3500 EMIT ® A.E.D. method instruction sheet.

Book Reviews

Essential Haematology. A. V. Hoffbrand and J. E. Pettit, 1980. Published by Blackwell Scientific Publications. Two hundred and forty-eight pages, 124 illustrations. Paper. NZ$27.35. Available from Blackwell Scientific Book Distribution Pty Ltd, 214 Bankeby Street, Cavltai 3053, Australia.

This textbook, presented in paper-back form and available at a reasonable price, is designed as a summary of current Haematological Practice for clinical students, medical laboratory workers and general physicians. The text is divided into 14 chapters: Blood cell formation; Iron deficiency and other hypochromic anaemias; Megaloblastic anaemia and other macrocytic anaemias; Haemolytic anaemia; Aplastic anaemia and anaemia in systemic diseases; The white cells; The Leukaemias; Malignant lymphomas; Multiple myeloma; Myeloproliferative disorders; Platelets, blood coagulation and haemostasis; Bleeding disorders due to vascular and platelet abnormalities; Coagulation disorders; and Blood transfusion. Each chapter concludes with a selected bibliography of references, the majority of which are taken from material published between 1976 and 1980, and the volume concludes with quite a comprehensive index.

One of the features of this textbook is the clarity of the figures and tables presented. In many texts these can be complicated and

confusing. The authors in this book have used both tables and figures to advantage. The graphs presented are simple and clear, and the black and white photos are of a most acceptable standard.

This is a very readable book, up to date, concise and lucid, and while not attempting to replace the basic haematological texts, certainly has a place on any Haematology Department bookshelf. I can see this textbook having especial value for both tutors and students preparing for Part II and Part III haematology examinations.

— E. R. Crutch.

Understanding Medical Terms. Ralph Rickards. Publisher: Churchill Livingstone. One hundred and six pages, soft backed. Price: NZ$15.40 from N. M. Peryer, Christchurch.

This book is a self-instructional course for those who wish to learn the meanings of common medical terms. The author shows how medical terms are derived from Greek and/or Latin roots plus suffixes or prefixes and how if we know the meaning of these, we can deduce the English meanings of the medical words. It is divided into sections including: the Body, Prepositions, Adjectives, Disease and Surgical Procedures with a short test at the end of each section.

There are many excellent annotated diagrams and the text is kept simple, enabling one to work through the book, learning more complicated terms on the way. It should enable new trainees in the laboratory to understand medical terms that are usually "picked up" on the job.

— S.G.

Biochemistry. By L. Stryer, second edition, W. H. Freeman, San Francisco, 949 pages. Illustrated. Publication date 1981. NZ$31.50. Available from N. M. Peryer, CPO Box 833, Christchurch.

The author of this book teaches medical students at Yale and Stanford and the quality of the production reflects the standing of these institutions.

As the aim of a book of this nature must be to be used as a teaching text the layout must be such that all relevant information stands out. The production team must be congratulated on the manner in which this is achieved; for example the Table of Contents is followed by a List of Topics which subdivides each chapter making the finding of information under general headings an easy task.

This is one of a very few books at this price level which uses large amounts of colour to illuminate vital facts in the plentiful diagrams. These diagrams are located on the same page as the relevant text which avoids the nuisance of having to seek out diagrams which mars so many other texts.

Important definitions are included in the wide margins of the pages whilst less important matter is italicised in the text. These features make for ease of revision and for a considerable saving of time in note taking.

The book is divided into five main divisions which cover the main topic of Biochemistry. Each chapter is followed by a series of questions with answers often amounting to 100 words at the end of the book.

There are also guides to where further information may be found, references to original works and useful sources of information.

The index is sufficiently detailed and with the help of the List of Topics any subject matter can be readily found.

The content of the individual divisions is well organised. The section on Information is almost a book in itself.

I can recommend this book to the serious student. To the medical technologist working in any discipline it would be a worthwhile purchase.

— W.H.M.

Basic Principles of Cancer Chemotherapy. K. C. Calman, J. F. Smyth, M. H. M. Tattersall. Macmillan Press Ltd. One hundred and fifty-eight pages. $22.60. Available from Macmillan Co. of N.Z., P.O. Box 33-570, Takapuna, Auckland 9.

Although not strictly a laboratory orientated book, "Basic Principles of Cancer Therapy" provides an easily understood and easy to read text on this subject. Trainees who are required to study chemotherapy for part of their syllabi would find this book useful as some of the chapters include Mechanism of Action of Anti-Cancer Drugs; Principles of Chemotherapy; Staging, Evaluation of Treatment and Prognostic Factors; and Drug Interaction in the Treatment of Neoplastic Disease.

There are also chapters introducing the reader to basics of tumour biology and biochemistry. Although it is not specifically intended to cover a particular cancer, the haematological malignancies are covered well, thus suiting the Part III haematology trainee. With this in mind Basic Principles of Cancer Chemotherapy is recommended particularly for the Haematology Laboratory.

— J.E.L.

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Contents

Medical Science

QUINICRINE BANDING (Q-BANDS) OF HUMAN CHROMOSOMES
D. R. Romain 41

PARASITIC ZOONOSES IN NEW ZEALAND
P. F. L. Boreham 44

CAMPYLOBACTER SEPTICAEMIA — A CASE STUDY
Mrs Anne Paterson 48

THE SEPARATION OF SERUM IgM BY GEL-FILTRATION FOR USE IN TOXOPLASMA INDIRECT IMMUNOFLUORESCENCE
Ray T. M. Cursons and Sherryn Cepulis 50

A NEW EOSINOPHIL DILUTING FLUID
J. E. Lucas 53

BLOOD GLUCOSE MONITORING: DEXTROSTIX AND BOEHRINGER TEST STRIPS COMPARED IN A SEMI-QUANTITATIVE AND QUANTITATIVE ROLE
R. McKenzie 54

"RALPH" GLASS KNIVES — THEIR EFFECT ON PARAFFIN AND RESIN SECTIONS
Glenys Rothwell 59

HALOPHILIC VIBRIO ISOLATES FROM THE EAR
Kay P. Bramley, B. M. Cornere 62

GILFORD 3500 — REDUCTION IN REAGENT VOLUMES WITH EMIT® TECHNIQUES 64

Book Reviews 65

INDEX TO ADVERTISERS

Carter Chemicals:
Transwab Transport System ..... Facing page 47

Carl Zeiss:
Zeiss Standard ..... Inside back cover

Ethnor Pty:
Visiting Lecturer 1.81 ..... Facing page 41

General Diagnostics:
Coag-A-Mate 2001 ..... Facing page 56

Northrop Instruments:
Wild Leitz 813 ..... Facing page 53

Philips Electrical:
PE 1248 ..... Inside front cover

Roche Products:
Toxoplasma ..... Facing page 46
Triglycerides Rapid ..... Facing page 47

Sci Med:
Breakthrough ..... Centre spread
IL System ..... Centre spread

Selby-Wilton Scientific:
Electrolyte AVL 980 ..... Facing page 52

Smith Biolab:
Radial Immunodiffusion 15040 .... Facing page 66
Sterile Vacutainer Tubes ..... Facing page 51

Technicon Equipment:
H6000 System ..... Outside back cover

Wellcome:
QCCC W8099 ..... Facing page 50

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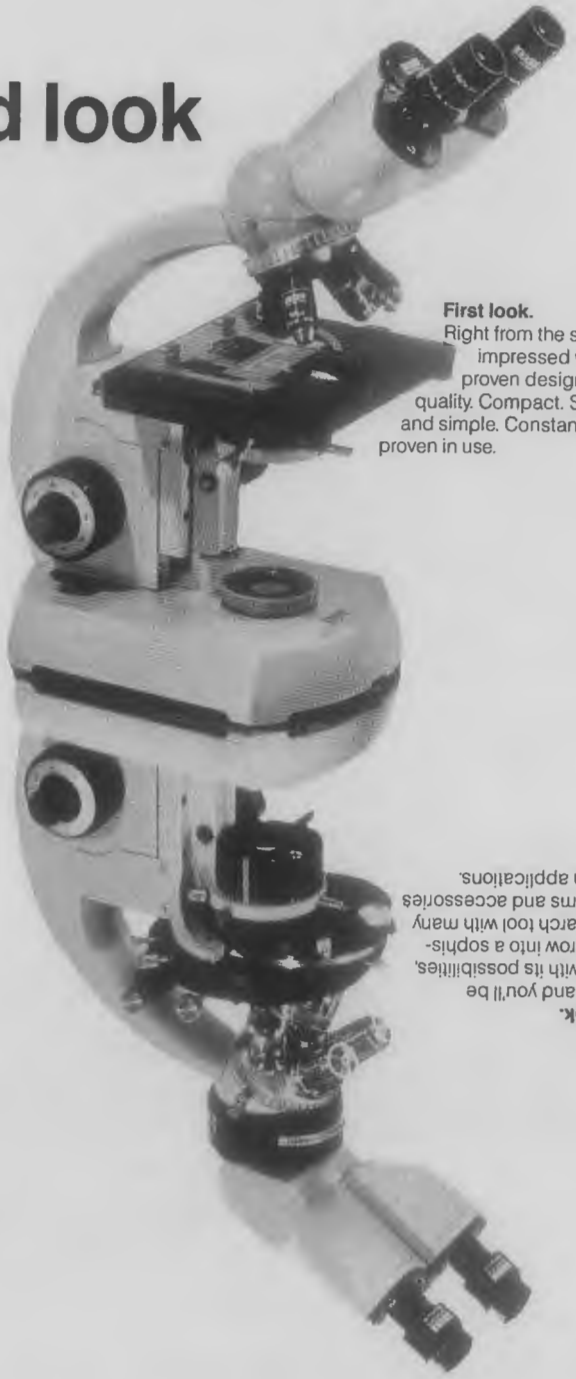

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