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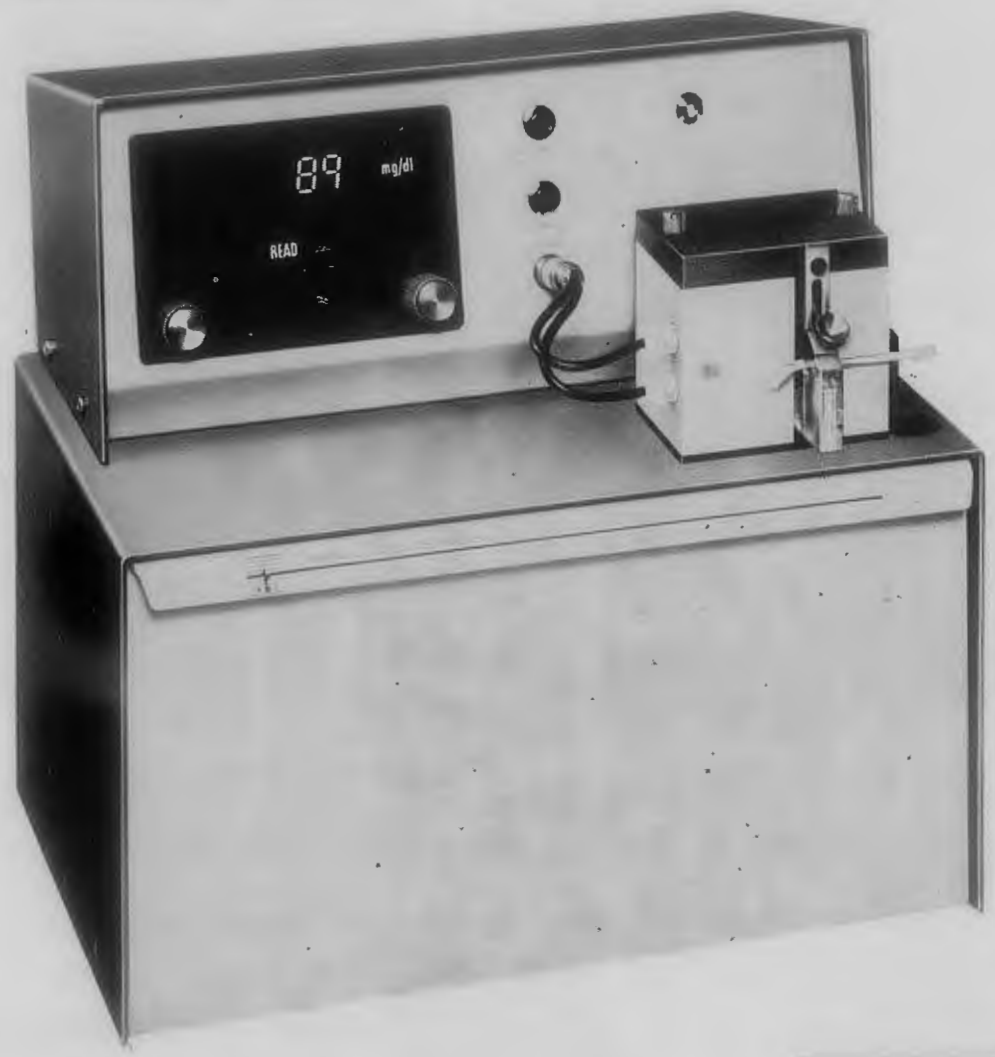

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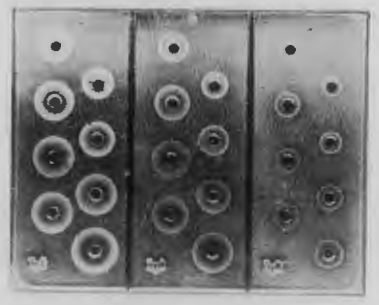

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A Cursory Review of some of the Myths Associated with Immunohaematology

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(A paper presented at the 31st Annual Conference of the NZIMLT, August 1975)

Since Landsteiner's discovery of the ABO blood group system, a great deal of knowledge has been amassed about human blood group antigens, their relationship to one another and the manner and mode of their inheritance. New and more sensitive techniques have been developed to facilitate the recognition, identification and quantitation of blood group antibodies, and immunohaematology has become a discipline of increasing significance in the practice of diagnostic laboratory medicine.

In the course of accumulating knowledge in the various facets of their subject, workers in blood grouping have progressively formulated and advanced new theories to account for their discoveries, in turn either adopting them when subsequent experiments seem to bear them out, or discarding them when they cannot be made to fit the experimental data. Aside from the controversy that still persists in regard to Rh nomenclature, there is surprising harmony amongst serologists in the interpretation of their findings; but sometimes ideas can become so deeply entrenched that they tend to persist long after agreement has been reached on their invalidity, and erroneous beliefs are often propagated in classrooms, when out-dated text books are the only source of inspiration to lecturers.

This paper examines a necessarily incomplete selection of imprecise ideas and mistaken notions, in an attempt to show that it is at least possible, when laboratory results fail to fit the "known" facts, that it could be the facts themselves which are in error, rather than the tests.

"Incomplete" Antibodies

This term was coined in the mid-1940's to explain why some antibodies failed to agglutinate red cells bearing the corresponding antigen when those cells were suspended (as was then the current practice) in physiological saline solution. It was recognised at that time that an antibody molecule required two antigen combining sites to participate in an agglutination reaction. Those which gave rise to such a reaction with saline-suspended cells were adjudged to be "complete" in this particular, whilst those which did not, except through the participation of a hypothetical entity called "conglutinin" which was to be found in bovine albumin solution, were seen as possessing only one antigen combining site, leaving them "incomplete".

When the structure of the immunoglobulin molecule came to be better understood, it was appreciated that "incomplete" antibodies belonged usually to the IgG class of immunoglobulins, which do in fact possess two antigen combining sites, but are disadvantaged by their size (by comparison with IgM molecules, which have ten antigen combining sites and the ability to span greater inter-cellular distances). Though long shown to possess the ability, under suitable environmental conditions, to join two cells carrying the appropriate antigen receptors, IgG antibodies are still referred to as "incomplete" today, for want perhaps of a suitably convenient and more correct term.

Zeta Potential

The phenomenon of agglutination with "incomplete" antibodies in a high protein environment was a source of speculation for some years, until Pollack advanced the idea that the distance separating red cells in suspension was a function of the zeta potential of the cells, which can effectively be reduced by the inclusion of albumin to an appropriate concentration in the incubating mixture. The effect of this, it was postulated, is to enable the cells to approach each other more closely than is possible when the cells are suspended in electrolyte solutions, thus enabling the IgG

molecules to form the necessary protein bridges to hold the cells in the three dimensional lattice we see as agglutination.

The ability of proteolytic enzymes to change red cells in such a way as to make them agglutinable by "incomplete" antibodies was believed by Wiener and Katz[10] to be due to the exposure of additional antigen receptors as a consequence of enzyme activity and, indeed, Wheeler *et al.*[9] and Masouredis[3] showed that enzyme treated cells adsorbed more antibody than untreated cells, though Hughes-Jones *et al.*[2] attributed this to an increase in the rate of association between antigen and antibody. Since, however, the treatment with proteolytic enzymes was shown to reduce the negative electrical charge at the red cell surface, the zeta potential explanation also served to account for the agglutinability of enzyme-treated cells by "incomplete" antibodies. In 1973, Stratton *et al.*[6] noted that zeta potential had little if anything to do with agglutinability. These investigators showed that the reduction in the negative surface charge on red cells could as effectively be achieved using neuraminidase as with papain, yet the former enzyme did not significantly improve the agglutinability of red cells by "incomplete" antibodies, whereas papain did so most effectively. Further experiments along similar lines were reported by Voak *et al.*[8], and the theory as it stands at present suggests that albumin achieves its effect through adsorption by adjacent cells, whilst enzymes cause clustering of antigen receptors, or else reduce steric hindrance by splitting polypeptide chains within the Rh antigen or surrounding it.

The Activity of Antibodies

Of considerable preliminary usefulness in the identification of antibodies is knowledge of the characteristics and physical behaviour of previously recognised examples of particular antibodies. Anti-Fy^a, for example, can normally be dismissed as a possibility if the antibody being investigated proves to be active by enzyme techniques, whilst most examples of anti-P_1 will be found reactive in saline and at temperatures below 30°C and Lewis antibodies will often lyse enzyme-treated cells carrying the appropriate antigen.

Helpful as such generalisations are, they should not be taken as inviolable rules. To dismiss the possibility, for example, that a saline-reactive antibody could be anti-Xg^a on the grounds that it would have to be active only by the indirect antiglobulin technique could be a serious error. Anti-Xg^a has, as it happens, been reported as a saline-reactive (and probably naturally-occurring) antibody on at least two occasions [1, 4] and witnessed, to the present author's knowledge, on at least one more.

Contaminating Antibodies in Standard Antisera

When a standard typing reagent gives a positive reaction with a test red cell suspension it can normally be assumed that the antigen corresponding to the antibody named on the label is present. The manufacturer will have taken the precaution of checking that his product does not contain antibodies to the common inherited antigens as contaminants, at least when it is used by the procedure recommended for the particular product's use; but it is always beyond his capacity to exclude the presence of antibodies directed at less common antigens. Antibodies to so-called "private" antigens occur commonly in normal sera, and in the sera of immunised subjects they are even commoner, not to mention the Bg antibodies, which are an endless cause of trouble.

In a recent case at Wellington, the cells of a laboratory staff member were found to be reactive with a commercial anti-C^w serum, although the cells concerned appeared to be Rh (C and E) negative. Further investigations with other anti-C^w sera failed to show any positive reactions, and it is plain that the original reagent contained an additional antibody besides anti-C^w, which had not been suspected by the manufacturer and would not have been suspected now had the cells being tested not given a negative reaction with anti-C. The contaminating antibody has still not been identified, but this is hardly surprising because the corresponding antigen is obviously one of very low incidence, and there are many such. The case illustrates, however, the desirability, perhaps, of performing all typing tests in duplicate, using two different reagents of each specificity.

Seemingly "Different" Antisera

The only trouble is that anti-sera of separate batches, or even from separate manufacturers, cannot invariably be guaranteed as being from different donors. There is an international

Anti-C (rh′)	Anti-D (rho)	Anti-E (rh″)	Anti-c (hr′)	Anti-e (hr″)	Anti-C^{w} (rh$^{w\,1}$)
—	—	+	+	+	+

Table I.—Preliminary Rh typing reactions on the blood of Mrs M.S. The two separate "anti-C" reagents represented in the first column were in reality anti-Ce (anti-rh$_i$), though supplied commercially as anti-C (anti-rh′). Each could be made to react by applying an enzyme technique, but reaction did not develop within the incubation time specified in the manufacturer's leaflet.

chain of agencies which traffick in donations of serum or plasma from persons who have formed antibodies potentially useful as typing sera. All manufacturers of typing reagents draw on these agencies from time to time for their supplies of raw material.

A few years ago, during the investigation of a Chinese family in which there were three separate exclusions of paternity (which as it turned out were erroneous and due to the contamination of an anti-S reagent with an antibody directed at a satellite antigen of the MNSs system), five separate anti-S reagents, all obtained from different commercial sources, were found by Sturgeon *et al.*[7] to be giving the false positive reaction. Enquiry revealed that all five were in fact from the same donor.

Typing Reagents Labelled Misleadingly in Regard to their Specificity

Sometimes a standard typing reagent may fail to react with a cell suspension in which the antigen corresponding to the antibody named on the label is actually present. The phenomenon occurs more commonly than may be realised with commercial anti-C reagents, and is potentially a source of serious error, particularly in cases of disputed paternity.

On preliminary testing, the cells of a patient investigated recently in Melbourne gave the reactions shown in Table I.

Without the positive anti-C^{w} reaction the patient's phenotype would have been reported without question as ccddEe, but in the circumstances tests were undertaken with additional anti-C sera and were found to be giving a weakly positive reaction with some of them.

At that point a sample was referred to the author's laboratory, where tests with many anti-C reagents revealed that a normal strong positive reaction (equal to that seen with Ccddee cells) was obtained with anti-C sera produced in Rh negative subjects (and containing incomplete anti-D). "Anti-C" produced in immunised Rh positive subjects, however, invariably gave either an unequivocally negative reaction, a perceptibly weaker reaction than was seen with normal C+ cells, or else required a prolonged period of incubation coupled with a measure of subjective judgment to be seen as giving anything approaching a positive reaction at all. Further additional tests gave the reactions shown in Table II.

Anti-C (rh′)	Anti-Ce (rh$_i$)	Anti-CE (rh)	Anti-ce (hr)
+	—	+	+

Table II.—Subsequent re-typing reactions on the blood of Mrs M.S.

Plainly the patient's Rh genotype was $r^{y^{w}}r$, and the original false negative or weak reaction with several "anti-C" reagents from different commercial sources was due to the fact that most such reagents are in reality anti-Ce (rh$_i$) and not anti-C (rh′), as all were clearly labelled. Those which did react contained an anti-C component, but in each case this gave demonstrably weaker agglutination with cells representing the C antigen as the product of an R^{z} or r^{y} gene complex, by comparison with the C antigen produced by R^{1} or r'.

It is inherently a problem with "anti-C" produced in immunised Rh positive donors that it contains the bulk of its activity in the form of anti-Ce. Though an anti-C (or perhaps anti-CE) component is virtually always present in addition, its potency is invariably inferior and this activity is not uncommonly lost altogether as a result of dilution in the course of manufacture, though the final product is still blithely labelled as anti-C (rh′) when it should more correctly be offered as anti-Ce (rh$_i$). Those reagents which give a demonstrably weaker reaction with CE cells are perhaps entitled to be labelled anti-C, but the accompanying leaflet should caution that weaker than normal reactions are to be watched for and their significance explained.

Quantitative Effects

On occasions a standard antiserum may give a weaker reaction with a test cell suspension than is normal for cells carrying the appropriate antigen. Depending on the intelligence, perceptiveness, experience or competence of the observer, this weaker than normal reaction may be recognised as such, taken as being the same as a normal positive or not recognised at all. In turn, depending on the same qualities, the significance of a weaker than normal reaction will be correctly interpreted, incorrectly interpreted or no attempt will be made at interpretation. Relevant additional tests may or may not be carried out, and the sample may or may not be referred for evaluation to a reference laboratory.

Commonly enough, the application of a more sensitive technique may be necessary to detect weaker than normal antigens. A case in point is the example of $r^{w}r$ cells previously mentioned, which were in fact reactive with all sera purporting to be anti-C if an enzyme technique were applied, though they failed to react when most of the reagents were used by their respectively recommended techniques. Such enhancement of sensitivity is fraught with obvious problems, of course, as the reactions thus obtained may not necessarily be specific ones, but in the case mentioned the tests were carefully controlled and were plainly significant, being due to small amounts of genuine anti-C not active by the regular procedures.

The D^{u} Antigen

Perhaps the most commonly encountered example of a quantitative effect is the D^{u} antigen of the Rh system. Much mythology is associated with this entity, which is usually defined as being a variant of the D antigen that is detectable with some but not all anti-D typing reagents. D^{u} seems to exist in various grades, from the so-called "high-grade" D^{u}, which may not show a markedly weaker reaction with most anti-D sera, to the so-called "low-grade" D^{u}, which is said to be capable of detection only by the application of the indirect antiglobulin test using selected anti-D sera.

The definitions were formulated before the advent of modern reagents and are perhaps no longer entirely true. With the highly potent rapid slide-test reagents in use at the present time, even the "low-grade" variant usually gives a detectable reaction in a slide test, though this invariably appears perceptibly weaker than that seen with the same serum against "normal" D cells. "High-grade" D^{u} probably goes unrecognised altogether in most instances.

The strength of the reaction observed between a slide-test anti-D reagent and D+ cells is much dependent on factors other than the actual potency of the reagent itself. It has been noted that the concentration and condition of the test cells suspension and the temperature and duration of incubation, as well as the attentiveness and visual acuity of the observer are all factors that come into play, and it was proven in the 1973 Serology Survey of the Royal Australian College of Pathologists that a single example of a D^{u} cell suspension can separately, in different laboratories, be equally easily reported as D+, as D— or correctly recognised as D^{u}. Such variation can even occur on different occasions in the same laboratory, as the factors mentioned are seldom in practice kept invariably constant.

The Imperfection of Control Tests

The case of the r^{w}r patient, again, illustrates how a false sense of security may be engendered when positive and negative controls are run in parallel with tests proper. A clearcut strong positive reaction with control cells representing the genotype $r'r$ when a serum purporting to be anti-C (but in reality being anti-Ce) is being used, will lend strong false confidence to the recording of a negative reaction with cells representing the products of R^{z} or r^{y}.

Similarly the only commonly used control in direct and indirect antiglobulin tests, could give rise to a feeling of false confidence. The fact that the reagent being used gives a reassuringly strong reaction when cells coated with an Rh antibody are added gives no indication that complement-binding antibodies are necessarily capable of detection with that particular reagent. Nor, for that matter, is the detection of other IgG antibodies (such as anti-Fy^{a}) necessarily assured. There are obviously limits to the extent of controls that can realistically be applied, but it is well to be aware of the limitations of such controls as are practicable, and to apply additional ones where these are crucial.

Conclusion

From this review it is apparent that the authority of the printed word is not necessarily

absolute. Whether concerning text books or the labels and leaflets that accompany reagents, it would be wise not to ascribe to them the authenticity of Holy Writ.

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A Survey of Pasteurella Multocida in the Oral Cavities of Cats

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Summary

A survey to determine the incidence of the organism *Pasteurella multocida* in the mouths of cats was carried out. Of 199 animals examined, 84 (42 percent) gave a culture of the organism. Six of the 84 isolates were identified as *Pasteurella n.sp. I* or *"gas" (multocida?)*. Twenty-three of the isolates were tested for their sensitivity to antibiotics. None of the isolates showed resistance to any of the antibiotics tested.

Introduction

Like other members of the *Pasteurella* genus, *P. multocida* is a Gram-negative, non-motile cocco-bacillus which shows bipolar staining. It is described in Topley and Wilson's "Principles of Bacteriology and Immunity" (5th Edition)[18] as having relatively slight powers of anaerogenic carbohydrate fermentation. The organism is primarily a pathogen of a variety of lower mammals, where it is the causative agent of haemorrhagic septicaemia. For many years, the *Pasteurella* isolates from cases of haemorrhagic septicaemia were named according to the infected host species: viz., *P. muriseptica* in rodents, *P. suis-septica* in swine, *P. boviseptica* in cattle, etc. It was later shown that these were different biotypes of the same organism associated with diseases in the various animals. These were then grouped together under the specific name *P. septica*. This was later changed to *P. multocida*. The organism is closely associated with contagious nasal catarrh or "snuffles" in rabbits and is also the causative agent of fowl cholera. In New Zealand veterinary practice, the organism has also been isolated from cases of feline pneumonia Carter (1972)[3], bovine pneumonia Carter (1972)[4], abortions in swine Carter (1972)[4] and bovine mastitis Carter (1974)[5].

Apart from its role as a potential animal pathogen, the organism has also been recognised as a member of the "normal flora" of the upper respiratory tract and mouth in a variety of both wild and domestic animals.[1, 9, 14, 17].

As a consequence, a number of human infections due to *P. multocida* can be traced directly to an animal source.[14, 17] In some cases the animal involvement is less obvious, although there appears to be a close relationship between the disease process in the patient and an association with animals.[10, 15] *P. multocida* is a versatile human pathogen and has been isolated from cases of meningitis Repice *et al.* (1975)[15], respiratory tract infections Miller (1963)[12], panophthalmitis Galloway *et al.* (1973)[7],

septicaemia Normann *et al.* (1971)[13] and osteomyelitis Burns *et al.* (1959)[2]. The organism frequently gains entry into the host by means of infected wounds resulting from animal scratches[6, 8, 9, 17] and bites. [1, 2, 8, 9, 11, 13, 17]

In the literature cited above, there is a large body of accumulated evidence which incriminates the cat as one of the most common sources of the organism. Because of this, the present survey was carried out to ascertain the incidence of *P. multocida* in the mouths of cats. There have been surveys of a similar nature in other countries, [8, 9, 14, 17] but to the author's knowledge, no previous investigations have been reported in New Zealand.

Materials and Methods

199 cats were examined for the presence of *P. multocida* during an 18 weeks period (16 October 1973-27 February 1974). The animals were located at the Wellington Branch of the SPCA and at the Tasman Street Veterinary Clinic, Wellington. Both facilities have a high turnover of animals and between them offer a representative portion of the Wellington feline population. None of the animals included in the survey showed any obvious infection of the oral cavity.

The oral microflora of the animal was sampled by passing a dry swab into one side of the mouth and then gently brushing the gums of both upper and lower jaws. Once the swab was thoroughly moistened by the animal's saliva, it was inoculated directly on to a 5 percent human-blood-agar plate. A mass inoculum was made along one side of the plate. On returning to the Institute, the mass inoculum was streaked for single colonies.

The plates were then incubated aerobically at 37°C for 18 hours.

Suspect *P. multocida* colonies were tested for oxidase activity, Gram-stained and stained with Loeffler's Methylene Blue. Colonies giving appropriate results for these tests were then inoculated into peptone water to which a few drops of either serum or yeast extract had been added. These were incubated at 37°C until turbid, usually 4 - 6 hours. One percent peptone sugars and a urea broth were then inoculated with a pasteur pipette. A MacConkey slope was inoculated to check for growth on this medium and a blood agar plate was also inoculated to check the purity of each inoculum.

The biochemical reactions were read after 48 hours at 37°C.

Antibiotic sensitivity testing was carried out by the method described in the Association of Clinical Pathologists Broadsheet Number 55. Stokes *et al.* (1972)[16]. The medium used was Oxoid DST. The control organism was the Oxford Staphylococcus NCTC 6571.

The antibiotics tested were:

Penicillin	2 units	Chloramphenicol	30 μg
Ampicillin	10 μg	Streptomycin	25 μg
Tetracycline	10 μg	Trimethoprim	1.25 μg
Cephaloridine	5 μg	Sulphadimidine	250 μg

Four antibiotics were tested on each plate. The trimethoprim and sulphadimidine discs were placed side by side, approximately 15-20 mm apart, to observe if the combination was synergistic for the organism. Twenty-three of the isolates were tested.

Results

Of 199 cats examined, 84 yielded cultures of *P. multocida*. After 18 hours incubation on the primary blood agar plates, the colonies were round and convex with an entire edge. They were approximately 1 mm in diameter and had a pearly-grey lustre. None of the isolates showed either alpha or beta haemolysis. All isolates had the same odour when cultured on solid media. The odour is reminiscent of *Haemophilus* sp. but is rather stronger. All isolates were oxidase positive (within 20 secs) and were Gram-negative coccobacilli which showed bipolar staining when stained with Loeffler's Methylene Blue.

Seventy-eight of the isolates produced acid only in sucrose and mannitol and were urease negative. The other six isolates were negative in mannitol and were urease positive. All 84 isolates were strongly positive for indole production, none grew on the MacConkey medium. It was thought that the six isolates that differed from the rest were *P. pneumotropica*. However, further biochemical tests showed this to be erroneous. Two of these isolates were referred to the Center for Disease Control, Atlanta, U.S.A., and were subsequently identified as *Pasteurella n.sp.I* or *"gas" (multocida?)*. The essential differences in the biochemistry of this organism, that distinguish it from the other *Pasteurella* spp., are shown in Table I.

Table I * — Distinguishing biochemical characteristics of *P. multocida, Pasteurella n.sp.I* or *"gas" (multocida?)* and closely related *Pasteurella* spp.

	P. multocida	*P. "gas" (multocida?)*	*P. ureae*	*P. pneumotropica*
Oxidase	+	+	+	+
Indole	+	+	—	+
MacConkey	No growth	No growth	No growth	No growth
Mannitol	A	—	A	—
Sucrose	A	A	A	A
Xylose	A	—	—	A
Ornithine	+	—	Not done	+
Urease	—	+	+	+

Key
+ = a positive result
— = a negative result
A = acid only in carbohydrate fermentation
* From King's Tables: Weaver *et al.* (1972)[19]

Antibacterial Sensitivities

None of the 23 isolates showed resistance to any of the eight antibiotics. Using Stoke's criteria for the reporting of sensitivity results — five of the isolates were only moderately sensitive to Penicillin and two others were only moderately sensitive to Sulphadimidine. One of these was only moderately sensitive to Trimethoprim. All other isolates were sensitive to the eight antibiotics.

Synergy between Trimethoprim and Sulphadimidine could be shown when two discs were tested on the same plate instead of the usual four. This was due to the fact that the discs gave large zones of inhibition with the test organism, and any increase in zone size due to synergy caused a merging of zones from opposing discs. The difficulty was overcome by dividing the plate, and inoculating one side with the test organism and the other with the Oxford Staphylococcus. The two discs were then placed 15-20 mm apart. In this manner a synergistic effect was readily demonstrated.

Discussion

The results of the survey show that 84/199 (42 percent) of the cats tested were carriers of *P. multocida.* In surveys carried out by Hawkins (1969)[8], Owen *et al.* (1968)[14] and in a survey cited by Hubbert *et al.* (1970)[9], the incidence was 21/50 (52 percent), 47/67 (70.2 percent) and 67 percent — respectively. Hawkins (1969)[8] took specimens from two sites, the tonsils and the gums, this would possibly increase the rate of isolation. Initially it was intended to sample from two sites, the second being the animal's claws. However this was hazardous when working alone and it was therefore abandoned. It is probable, however, that the claws of cats would yield cultures of the organism because of their habit of licking their claws whilst cleaning themselves. Isolation of the organism from the cats' claws would correlate with the large number of infections following cat scratches as cited in the literature.

Because of their widespread popularity as pets and their tendency to bite or scratch, the cat must constitute a major reservoir of human *P. multocida* infection in New Zealand.

Cat scratch fever in man should be differentiated from *P. multocida* wound infections. The aetiological agent in the former condition has been suspected to be a Chlamydia. Cat scratch fever is usually self-limiting, but the primary lesion following injury often leads to lymphadenitis and associated fever.

Physicians and medical laboratory technologists should be aware of the variable manifestations of the pathogenicity of the organism. While the association with infected animal bites and scratches is well known, the significance of an isolation from a patient without a history of animal trauma is not always appreciated. Hubbert *et al.* (1970)[10], in a study of the epidemiology of *P. multocida* infection unrelated to animal trauma in the United States, showed that the most common sites of infection in order of frequency were: the respiratory tract, abdominal disorders, infections involving the extremities, the CNS and less common infections including septicaemia and eye infections. To further illustrate the variable pathogenicity, data regarding nine isolates submitted to the

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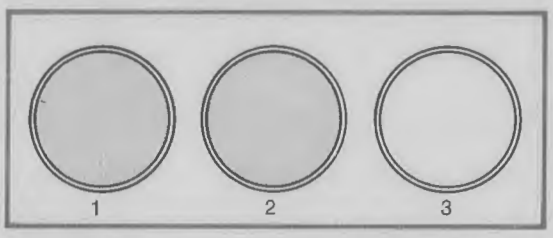
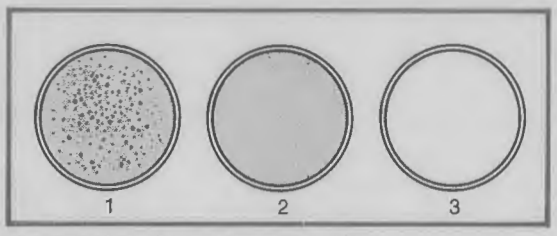
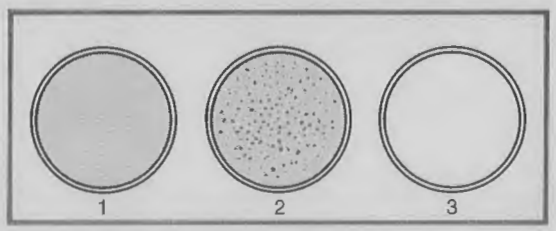


No agglutination in either test circle: **negative** for Infectious Mononucleosis. Absence of agglutination in both circles can also indicate a **positive** test for serum sickness antibody, since both absorbents neutralize this antibody. To confirm the presence of serum sickness antibody, test one drop of patient sample with one drop of MONO-DIFF TEST Reagent, without absorbents. If agglutination is observed, the test is **positive** for serum sickness.



Stronger agglutination in circle 1 than circle 2: **positive** for Infectious Mononucleosis.



Stronger agglutination in circle 2 than circle 1: **positive** for Forssman antibodies, **negative** for Infectious Mononucleosis.

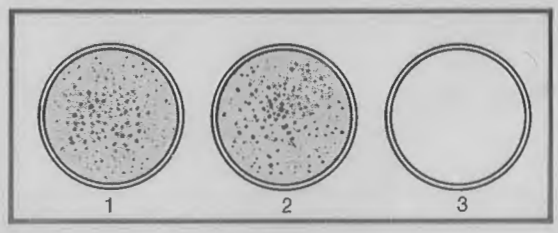


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Table II — Data relating to nine isolates of *P. multocida* submitted to National Health Institute for confirmation during the period May 1971 - May 1975.

Patient	*Year*	*Sex*	*Age*	*Site*	*Clinical Data*
1	1971	M	2/12	Meninges	Meningitis
2	1973	F	17	Swollen infected hand	Infected cat bite
3	1974	?	?	Sputum	Pneumonia
4	1974	F	2	Left cheek	Infected dog bite
5	1975	F	41	Sputum	Bronchiectasis
6	1975	M	73	Sputum	Cancer of larynx
7	1975	M	50	Perianal abscess	Diabetic
8	1975	M	?	Sputum	Chronic lung disorder
9	1975	M	?	Left hand	Infected dog bite

National Health Institute for confirmation is given in Table II.

In Table II it is apparent that only three patients (Nos. 2, 4 and 9) derived their infections from animal trauma. The remaining six have infections which were unrelated to obvious animal trauma. Such non-traumatic infections may possibly result from the contamination of human skin either by being licked by an animal, or by stroking recently self-groomed animal fur.

The *P. multocida* isolates were readily identified apart from the six isolates of the organism designated in King's tables (Weaver *et al.* 1972)[19] as *Pasteurella n.sp.I* or *"gas" (multocida?)*. The essential differences between this species and other *Pasteurella* spp. are given in Table I. An isolation of the latter organism should be confirmed by the reference laboratory at the National Health Institute. It is of interest that the present six isolates were the first confirmed in New Zealand. More recently, another isolate (No. 7, Table II) was submitted to the N.H.I. and confirmed as *Pasteurella n.sp.I* or *"gas" (multocida?)*.

The production of gas in the fermentation of glucose is a variable property of this organism. None of the New Zealand isolates have shown this feature.

The results of the antibiotic sensitivity tests show that the organism is sensitive in vitro, to most commonly used antibiotics. This is in agreement with the findings of other investigators. [1, 7, 11, 12, 13, 15, 17] The organism's sensitivity to Penicillin is of special interest as it is one of the few Gram-negative bacilli that are sensitive to this antibiotic. This property may assist identification of the organism in the medical laboratory, but it cannot be used as the sole criterion.

In *P. multocida* infections, as in all other infections, the technologist must be guided by the case history, where one is given, and by what is seen in a direct Gram-stain of the specimen. *P. multocida* appears as Gram-negative bacilli/cocco-bacilli in Gram stains and may be confused with *Haemophilus* spp. in a sputum. Both *Haemophilus* spp. and *P. multocida* will grow on chocolate agar (or similar), but the use of a 10-unit disc of Penicillin placed on the mass inoculum for easier recognition of *Haemophilus* spp. will have the reverse effect with *P. multocida*. The unusual sensitivity of *P. multocida* to this antibiotic makes it unsuitable for this purpose, however a Bacitracin disc will enable easy recognition of both bacilli on culture.

P. multocida is distinguished from *Haemophilus* spp. by its growth in vitro in the absence of X and V factors. The production of indole and the reduction of nitrates set it apart from the *Acinetobacter* (formerly *Mima*) and *Neisseria* genera. It may be distinguished from members of the *Enterobacteriaceae* by virtue of its positive oxidase and its inability to grow on media containing bile salts.

It would be of interest to determine if the organism can be isolated from the cat's claws, since, to the author's knowledge, no surveys have been made with specimens from this site.

The association of the organism with dog bites in two of the nine cases listed in Table II, suggests that a similar survey should be carried out on dogs in New Zealand.

Acknowledgments

The author is indebted to Dr R. A. Robinson, Microbiologist, and Miss D. Norris, Senior Bacteriologist, National Health Institute, for advice and helpful criticism in the preparation of this paper and to Miss M. E. Carter, BVSc of Ruakura Animal Health Laboratory for her communications.

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gratefully acknowledged.

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The Significance of an Increased Disc Content on Sulphonamide Susceptibility Tests of Gram-Negative Urinary Tract Infectors

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Received for publication, November 1975

Introduction

The development and use of soluble, rapidly excreted sulphonamide drugs for the treatment of urinary tract infection, questions the relevance of the results produced by the standard 250 μg sensitivity disc to the high urinary levels achievable. In an attempt to answer the question, 503 random Gram-negative isolates were tested for susceptibility to sulphamethizole, using discs with four different drug contents: 250, 500, 750 and 1,000 μg respectively. At the same time minimum inhibitory concentrations to these isolates were also performed and the results of the two tests were collated to assess the effects of an increase in the disc content.

Methods

503 isolates consisting of 226 *E. coli,* 125 Klebsiella species, 46 *Proteus mirabilis* and 106 miscellaneous organisms (Acinetobacter species, *Proteus vulgaris* and Enterobacter species) were tested by the following methods.

Disc Sensitivity

The method of sensitivity testing was a modification of the Stokes[9] method as described by Garrod and Waterworth 1971[4] and using discs containing 250, 500, 750 and 1,000μg of the agent. The modifications consisted of the use of pure cultures, and in the preparation of the inocula. The inocula were prepared by stabbing a 1 mm diameter colony of an overnight growth of a pure culture with a straight wire, until the surface of the agar was contacted. The wire was withdrawn and the organisms transferred to, and emulsified in 2.5 ml of sterile distilled water. This produced an inoculum of approximately 3×10^6 organisms/ml. A water diluent was used in order to wash nutrients from the organisms and to reduce the amount of pre-growth. Plates of Wellcotest agar 4 mm deep were prepared in plastic 9 cm Petri dishes without the addition of lysed horse red cells. These were inoculated with the prepared inoculum using a sterile throat swab. A similarly prepared inoculum of *E. coli* NCTC 9003 was also placed on each plate. One disc

of each strength was placed on every plate, and the plates incubated at 36°C without prediffusion. Results were recorded next morning after comparison of the zones produced with those of the control organism. Isolates showing a zone diameter equal to, or greater than half the zone diameter of the control, were recorded as sensitive, and those showing a zone less than half as resistant. Zone sizes were measured with a micrometer caliper. The 8 mm discs which each imbibed 20 μl of water on average, were specially prepared by Alpha Biologicals Ltd., Auckland.

Minimum Inhibitory Concentrations (MIC)

The MIC of the agent was determined for each isolate by means of agar dilution plates. Concentrations ranged from 1.8 μg/ml to 240 μg/ml in logarithmic increments and from there in 120 μg increments to 1,200 μg/ml. This produced a series of 16 steps. A change in increment at the 240 μg/ml level was used to increase the precision of the upper end of the series. Initial high concentrations of stock solutions were prepared in dimethyl formamide and diluted in alkaline water. 10 ml of a 10x concentration of each strength were added to 90 ml of molten and cooled agar to give the final concentrations. The standard suspension used for the disc diffusion was applied to the agar solution plates by means of a Clements Antibiotic Replicator (C. L. Clements Ltd, Sydney). This instrument delivers 32 spots to the surface of a 9 cm agar plate. Each spot consists of approximately 2×10^4 organisms and covers an area of 40 sq. mm. Each set of 32 organisms tested contained the control *E. coli* NCTC 9003, the MIC of which was 15 μg/ml. The effects of dimethylformamide at the concentrations encountered, on the growth of Gram-negative organisms were tested and found to be nil.

Results

1. *Disc sensitivities.* With the 250 μg discs, 83 percent of *E. coli* were sensitive, Klebsiella 47 percent sensitive, *Proteus mirabilis* 85 percent sensitive, and the miscellaneous group 75 percent sensitive. As the disc content was raised the number of sensitive strains increased, so that with the 500 μg disc the percentage of sensitive strains with the groups of organisms were 85 percent, 65 percent, 94 percent and 82 percent respectively. Similarly with 750 μg, the results were 87 percent, 76 percent, 96 percent and 83 percent. The organisms showing the most obvious change were the Klebsiella species. The distribution of the responses to the various disc strengths are recorded in Table 1. An analysis of the changes of each of the groups of isolates is shown in Table 3.

Table 1

		NUMBER OF ISOLATES SENSITIVE				
Organism	No.	250µg	500µg	750µg	1000µg	Res.
E.coli	226	187(83)	4(2)	4(2)	0	31(13)
Klebsiella sp.	125	59(47)	23(18)	14(11)	4(3)	25(20)
Pr. mirabilis	46	39(85)	4(9)	1(2)	1(2)	1(2)
Miscellaneous	106	79(74.5)	8(7.5)	1(1)	0	18(17)
Total	503	364(72)	39(8)	20(4)	5(1)	75(15)

Figures in parentheses show percentages.

Table 2

Minimum Inhibitory Concentrations

µg/ml	1.87	3.75	7.5	15	30	60	120	240	360	480	600	720	840	960	1080	1200
E.coli	1	0	5	53	78	32	16	10	0	0	0	0	0	0	0	31
Klebsiella	0	1	2	9	19	23	22	18	3	3	1	0	0	0	0	24
Pr.mirabilis	0	0	1	9	12	10	7	3	1	0	0	0	1	0	0	2
Miscellaneous	0	3	1	12	26	36	8	1	2	0	0	0	0	0	0	17

Figures represent numbers of isolates sensitive

Table 3

Identity of Isolates Changing Between 250µg and 750µg Discs

	Total	E.coli	Kleb.	Prot.	Misc.
Total No changing	59	8	37	5	9
% of No. sens.250µg	16	4	63	13	8
% of total change	100	14	63	8	15
% of total isolates	12	2	7	1	2

Table 4

MICs of Organisms Changing Sensitivity

	Mean	Mode	Range
Resistant at 250µg. sensitive at 500µg	120	120	30-480
" " 500µg " " 750µg	120	240	60-240
" " 750µg " " 1000µg	480	240	120-1200
		µg/ml	

2. *Minimum Inhibitory Concentrations.* The MIC produced typical bimodal distributions of organisms. There was clear separation of the sensitive from the resistant peaks in all instances (Table 2). The MIC of sensitive *E. coli* strains ranged from 1.87 μg/ml to 240 μg/ml, the peak being 30 μg/ml. With the Klebsiella sp. the figures are 3.75 μg/ml to 600 μg/ml with a flat peak covering 60 to 120 μg/ml. *Proteus mirabilis* ranged from 7.5 μg/ml to 360 μg/ml peaking at 30 μg/ml, and the miscellaneous groups ranged from 3.75 μg/ml to 360 μg/ml with the peak at 60 μg/ml. One isolated *Pr. mirabilis* strain was placed at 840 μg/ml.

Discussion

The amount of antimicrobial agent contained in the sensitivity disc is an arbitrary one, generally decided by the amount that gives a good zone by sensitive strains, yet one which still shows a significant reduction in the zone size by more resistant strains. The amount of sulphonamide used locally in sensitivity discs is 250 μg. The origin of this amount is difficult to establish. Evans[1] in 1948 described and confirmed a method originally published by Kokko[6] in 1947 in which blotting paper discs were impregnated with 0.05 ml of a 200 mg percent solution of sulphonamide were used. This gave a disc content of 100 μg. In this method the discs were placed on the uninoculated plate and allowed to diffuse for five hours. They were then removed and the organisms inoculated over the entire surface of the plate. Evans[1] also used 6 percent lysed horse blood as an additive in "ordinary peptone broths" to counteract the effect of sulphonamide antagonists. This technique was first described by Harper and Cawston[5] in 1945. The use of Wellcotest agar in this study circumvented the use of lysed horse blood (Garrod and Waterworth 1971)[4].

Later in 1954 Fairbrother *et al.* described a method in which the plates were inoculated first and the discs then applied, after the manner of the then increasingly common method for antibiotic sensitivity testing (Fairbrother and Rao 1954)[2]. These investigators employed 250 μg discs and compared their results with agar dilution MIC's. The disc content recommended by E. Joan Stokes (1968)[9] is 4,000 μg per disc which is considerably higher than any other mentioned in the literature. The introduction of rapidly excreted sulphonamides, with the resultant very high urinary concentrations poses the question whether the use of high potency discs would not better reflect the ability of these agents to control urinary tract infections. Sulphamethizole was used in this study as it is a relatively soluble drug which is rapidly excreted and yields very high urinary levels. 60 percent of the dose is excreted in five hours (Martindale 1972)[7]. Urinary levels up to 1,360 μg/ml are being recorded following a recommended dose rate of 2 × 500 mg tablets tds (unpublished data, Warner Medical Department). Such a drug might require high potency discs to reflect its true potential.

An analysis of this investigation reveals that an increase of the disc content results in an increase in the yield of sensitive organisms (Table 1). If these results are further analysed (Tables 3 and 4) it is found that the increases derive from those organisms with MIC's centring around the 240 120 μg/ml which were recorded as positive using the 750 μg disc. If urinary levels of 1,360 μg are achievable with sulphamethizole then the use of a 750 μg disc would appear to be acceptable. Some other factors should be considered however. On examination of the identity of those organisms reported as sensitive to the various disc strengths, it is immediately apparent that as far as *E. coli* is concerned the gain achieved by increasing the content from 250 to 750 μg is only eight isolates, a little over 3 percent. This organism accounts for the majority of urinary tract infections both inside and outside the hospital. The miscellaneous group similarly gains only nine isolates (8.5 percent). The bulk of those becoming sensitive are Klebsiella species, the figures showing a 29 percent gain for this organism. This fact requires that the attitude of clinicians to Klebsiella species as a urinary tract infector and to its treatment be assessed. On the results of such assessment would depend the value of increasing the disc content. Garrod regards all Klebsiella species as resistant in urinary tract infection (Garrod *et al.* 1973)[3]. On the other hand Stamey demonstrates sensitivity of Klebsiella species strains to sulphamethizole in 50 percent of strains tested (Stamey 1972).[8]

Conclusion

An increase in a number of potentially

sensitive organisms is achieved by raising the disc content of sulphamethizole from 250 μg to 750 μg for testing organisms derived from urinary tract infections. Increasing the disc content beyond this to 1,000 μg achieves little in an increase in the number of sensitive strains. The majority of those organisms making up the increase are Klebsiella species and this fact makes the usefulness of the procedure open to some debate. If the disc content was to be increased, the identity of the drug, in this case sulphamethizole, should be made clear on the report, to avoid any application of the result to the less rapidly excreted sulphonamides, or to the organisms isolated from sites other than the urinary tract.

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Diphtheria in the Waikato

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Microbiology Department, Waikato Hospital, Hamilton

A paper read at the NZIMLT Conference Palmerston North, 1975

Introduction

An outbreak of diphtheria is reported which occurred in the Waikato between August and November, 1974, and the laboratory findings commented on. It involved the isolation of 97 *Corynebacterium diphtheriae* by this laboratory, and 12 *C. diphtheriae* from medical laboratories, a private laboratory in Hamilton, which were recovered from 82 different people, three of whom were clinical cases of the disease, and 79 of whom were carriers.

Historical Background

In 1946 there were 1,577 notified cases of diphtheria in New Zealand, resulting in 49 deaths. Although immunisation was begun as early as 1922 — the first recorded instance of immunisation being in two Hamilton schools in that year — acceptance of immunisation was slow and it was not until about 1947 that it was well established, aided by the introduction of combined vaccines.[2]

Records kept at the Waikato Hospital which go back to 1948 show a rapid decline in admissions and deaths due to diphtheria in this period. In the period between 1948-52 there were 18 admissions and one death due to diphtheria, the same figures as for the last six months of 1947. Between 1958-62 there were five admissions and one death recorded for this disease, while between 1968-72 there were no admissions recorded for diphtheria[3]. There was one subsequent case diagnosd by the laboratory in January, 1974, from a patient with laryngotracheo bronchitis.

The remarkable decline in the incidence of this disease resulted in a situation where few of the laboratory staff were familiar with the recognition and isolation of the causative organism.

Clinical Cases

The initial case involved a 32-year-old male farmer from Paeroa. He had a history of sore throats, two or three times a year, sufficient to keep him off work for several days. He presented to his general practitioner with cervical adenitis in July, 1974. His physician noted a grey exudate over the left tonsil, took

a swab from this, then treated him with procaine, penicillin and co-trimoxazole.

The patient's condition improved rapidly but the swab which was sent to medical laboratories showed an organism morphologically consistent with the diphtheria bacillus, so the patient was admitted to Waikato Hospital.

All subsequent throat and nasal swabs were negative but the initial isolate was confirmed six days later by the National Health Institute as *C. diphtheriae var. mitis* — toxigenic strain.

Penicillin therapy, which had been continued since the patient was admitted, was discontinued after eight days and the patient discharged the following day.

The second case involved a four-year-old male child from Waharoa. He was taken to his general practitioner in August, 1974, with a history of a runny nose, coughing and wheeziness for four days. His physician treated him with oral ampicillin and sent him home. The patient returned the following day in a worse condition, so he was admitted to Waikato Hospital where he was found to have a temperature of 38.1°C, enlarged tonsils covered with exudate, respiratory distress and lung consolidation in the middle right lobe. A throat swab was taken and ampicillin therapy started.

The following day a typical membrane was present, diphtheria was queried and antitoxin given. The patient was intubated and copious secretions were aspirated by suction. The KLB from his throat swab was subsequently confirmed as positive and a toxigenic strain of *C. diphtheriae var. mitis* isolated from the specimen. The patient's condition gradually improved and therapy was changed to penicillin, which was discontinued after nine days. Three consecutive throat swabs taken after this were all negative so isolation was discontinued and the patient discharged in late September.

The third case was hospitalised and treated in Thames Hospital in September, 1974. She was from Paeroa.

Epidemiology

It was unfortunate that the first two cases of the disease occurred at the beginning of the August school holidays. The movement of children and families at this time made the tracking down and confining of contacts and carriers very difficult and undoubtedly assisted in the spread of the organism throughout the Waikato.

Figure 1

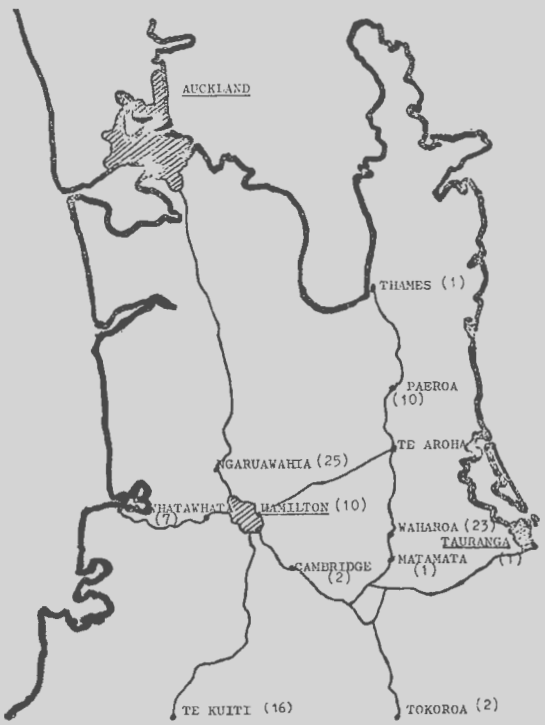

Figure 1. — Geographical locations of areas where *C. diphtheriae* were isolated. ( ) Number of people from whom *C. diphtheriae were isolated.*

The initial case directly infected one of his daughters but not his wife or his other two children. Although no direct link was established between them and the other positives in Paeroa, it is reasonable to assume the daughter introduced it at the school to which she and several other of the positives attended.

The movement of people between the Paeroa Pa and the Waharoa Pa appears to have been responsible for the introduction of the organism into the Waharoa area. *C. diphtheriae* was isolated from 23 people from this area and some of these people showed a remarkable degree of mobility around the Waikato during the time these investigations were proceeding. From Waharoa the organism was traced to Tokoroa, Matamata and Ngaruawahia. Positive contacts in the first two of these instances were limited, there being two positives from Tokoroa (one of whom was previously from Waharoa) and

3 volunteers went from lab to lab...

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*Lorberbaum, H.: Am. J. Clin. Path. *56*:487, Oct. 1971.

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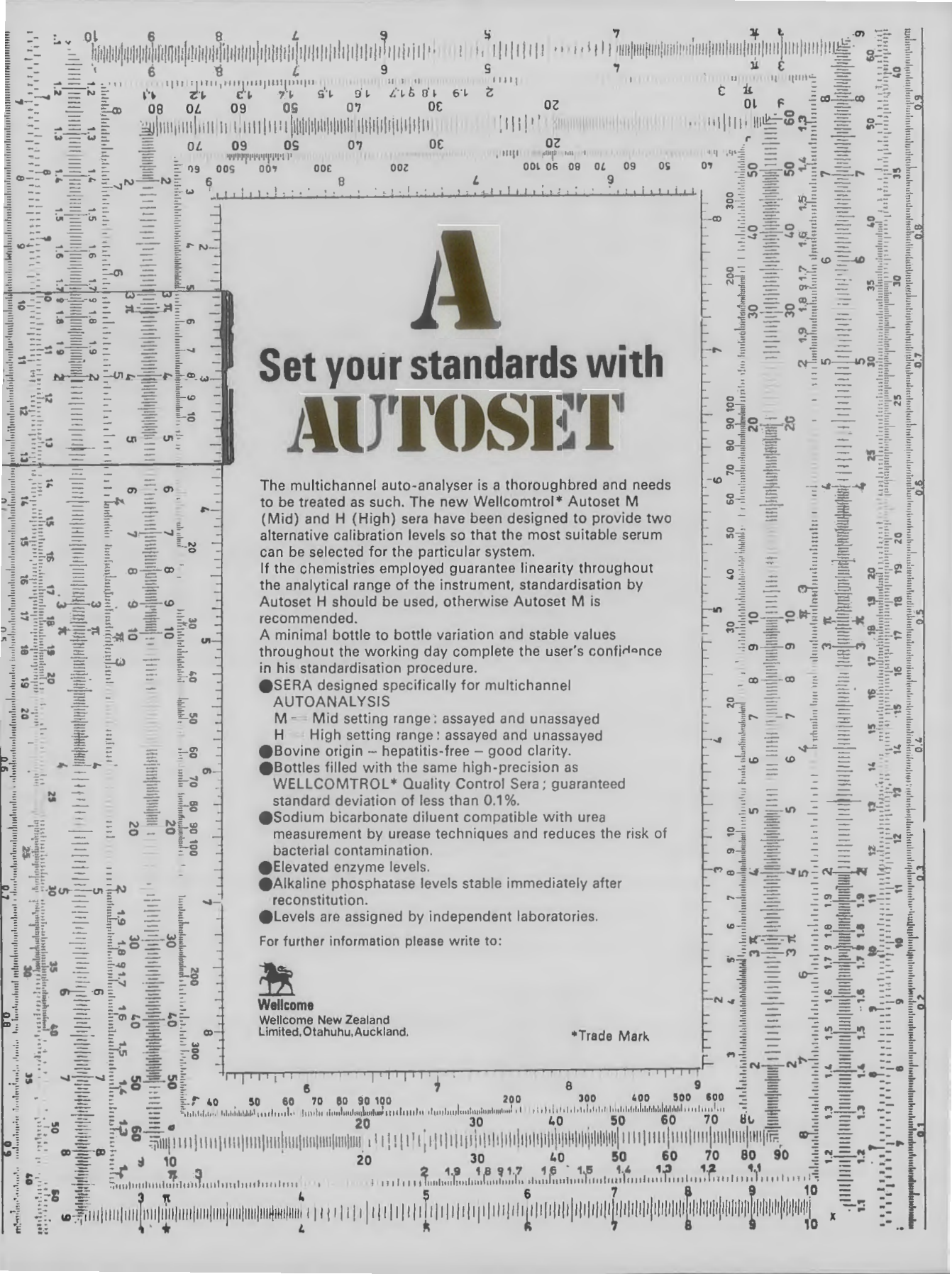

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Table I. — Distribution of isolates of C. diphtheriae.

	C. diphtheriae var. mitis			*C. diphtheriae var. gravis*	
	Toxigenic	Non-toxigenic	Not recorded	Toxigenic	Non-toxigenic
Paeroa	2	3	4*	—	1
Waharoa	9	14	—	—	—
Ngaruawahia	9	5	—	—	12
Hamilton	—	8	—	—	2
Whatawhata	4	3	—	—	—
Tokoroa	—	2	—	—	—
Cambridge	—	2	—	—	—
Matamata	—	—	—	—	1
Tauranga	1	—	—	—	—
Thames	1	—	—	—	—

* These people were seen by their general practitioners who notified the Health Department. Specimens from them were not received by this laboratory.

one from Matamata. There were also two positive isolations from Cambridge, although their occurrence there has not been explained. The introduction of the organism to Ngaruawahia is assumed to originate from a family who moved from Waharoa to Ngaruawahia at this time. In Ngaruawahia a large number of *C. diphtheriae var. gravis* were isolated and this made it difficult to establish whether positive contacts had a local strain or one that had been introduced into the area. There were also instances where individuals were found to be carrying both var. *gravis* and var. *mitis*. Isolations of *C. diphtheriae* were made in Hamilton from contacts of people in both Waharoa and Ngaruawahia. From Ngaruawahia, the organism was traced to Whatawhata where seven people were found to be carrying *C. diphtheriae*.

Two other isolations were made, one from Thames and one from Tauranga, but it is not known what contact, if any, they had with other known positives. This made a total of 109 isolations of *C. diphtheriae* from 82 different people and involved the Waikato Hospital laboratory in testing over 3,900 swabs from contacts as far away as Taupo, Rotorua and Christchurch. Of these positives a total of 10 people were hospitalised during this period. Apart from the cases already mentioned, one carrier was admitted to Waikato Hospital, two others to Thames Hospital and two to Tokoroa Hospital. The remaining two were both admitted to Waikato Hospital, one for tuberculosis treatment and one for encephalitis.

Microbiology

Initially, all specimens which required culture for diphtheria organisms only were inoculated on to a Loeffler's slope and half a Downie's medium plate. An Albert's stain for volutin granules was performed from the Loeffler's slope after 18 - 24 hours incubation at 37°C, and then from any growth on the Downie's plate after 48 hours incubation. Albert's stain was preferred to Neisser's stain because of the easier recognition of volutin granules found when using this stain. After a period of one month a total of 1,037 specimens had been tested in this way and it was found that the Loeffler's slopes had given 73 positive KLB's while the Downie's plates had given 267 positive KLB's. It was decided at this point to discontinue the Loeffler's slopes to avoid duplication of work and because subcultures were easier to obtain from Downie's plates.

Where typical polar volutin granules were present the organisms were subcultured into 1 percent peptone water solutions of lactose, glucose, sucrose, maltose, mannitol, dextrin and galactose, as well as on to a blood agar purity plate. Two drops of sterile ox serum was added to each tube and they were incubated at 37°C overnight. All positive KLB's were also subcultured on to Tinsdale's medium plates. Those catalase positive, Gram-positive rods showing acid production the following day in the glucose, maltose, dextrin and galactose tubes were further inoculated into starch and glycogen, and had toxigenicity tests performed on them. Those specimens showing negative sugar reactions were reincubated for a further 18 hours before being discarded as negative.

Table II. — Relative numbers and types of C. diphtheriae isolated.

	% isolation per population tested	*C. diphtheriae var. mitis* Toxigenic	Non-toxigenic	Not Recorded	*C. diphtheriae var. gravis* Toxigenic	Non-toxigenic
Outbreak	5%	26	37	4	—	16
Survey	3.4%	5	6	—	—	7
Routine Specimens	0.3%	—	6	—	—	7

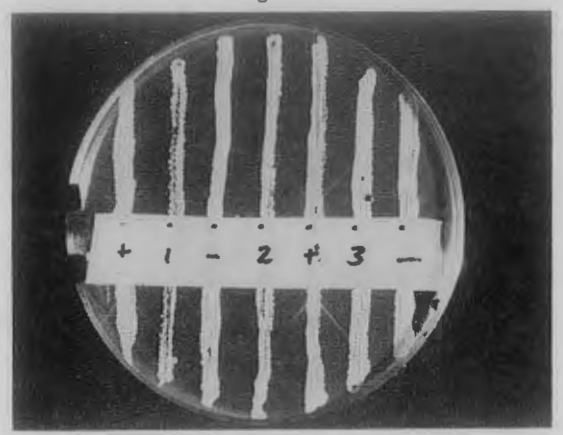
All isolates of *C. diphtheriae* gave typical reactions on Tinsdale's medium. This is a cystine-sodium thiosulphate-tellurite medium on which colonies of *C. diphtheriae* appear grey-black and are surrounded by a distinctly outlined dark brown halo. This halo is absent around diphtheroids or any other bacteria encountered in the human throat, except *C. ulcerans*.

Toxigenicity tests were performed according to Bickham and Jones modification of the Elek's test.[1] Control organisms were used with each isolate tested. Initially, dilutions of antitoxin of 1,000, 750, 500 and 250 units per ml were used but all of these were found to give non-specific precipitation lines with test organisms and negative controls. By extending these dilutions it was found that the best results were usually obtained using antitoxin dilutions between 100 - 200 units/ml and so dilutions of 500, 250 and 125 units/ml are now routinely used. The tests were interpreted as positive if, in the appropriate dilution of antitoxin, there was no precipitation lines with the negative control and single lines of precipitation were present between the positive control organism and the test organism and these lines met to form lines of identity (See Figure 2).

In retrospect, these procedures made our isolation techniques oversensitive. Certainly, using Albert's stain, volutin granules can be found in many organisms other than *C. diphtheriae*, especially from a Downie's plate after 48 hours incubation. Of the 3,852 Albert's stains performed during this period, 903 showed typical polar granules. Of these 903, only 97 were confirmed as being *C. diphtheriae*, the rest having negative Tinsdale's reactions and being shown by sugar fermentation tests to be mainly *Corynebacterium hofmannii* or *Corynebacterium bovis*. However, these organisms were easily screened out by the tests already mentioned.

Using these techniques, four isolations of *C. diphtheriae* have been made from routine throat and nasal swab specimens since this work was completed. All have involved unsuspected carriers of *C. diphtheriae var. mitis* -- nontoxigenic stains.

Figure 2.



Te Kuiti Survey

In November, 1974, the Health Department carried out a survey of primary school children in Te Kuiti to determine the carrier rate of *C. diphtheriae*. Te Kuiti was selected for survey purposes because it was in a "clean area" i.e., no previously positive contacts or carriers had been connected with this area. A total of 529 children were Schick tested and had throat and nasal swabs taken. Of this total, 30 children had a positive Schick test and none of these children had a positive swab. There were 18 *C. diphtheriae* isolates from the remaining children, eight from throat swabs and 10 from nasal swabs, two children having both throat and nasal swabs positive.

Discussion

The isolations of *C. diphtheriae* which were made indicate the degree to which this organism is still present in the community, and the potential threat it presents. The survey conducted on school children, who had no contact with the people from whom *C. diphtheriae* was previously isolated, showed that a significant proportion of the population continues to carry this organism and that where *C. diphtheriae*

var. mitis is isolated, a considerable number of these are toxigenic strains. This fact should be reflected by laboratory isolations, particularly from laboratories handling work from general practitioners. The remarkable difference in the isolation rates between those of the Te Kuiti survey and those found in routine specimens from hospital in-patients is not understood but may reflect the degree to which antibiotic therapy is used before hospital admission.

Acknowledgments

I would like to acknowledge the assistance given by Dr E. H. Gordon, Medical Officer of Health, both during and after this outbreak, and the technical work performed by Mrs S. Cepulis. I would also like to thank Dr C. M. David and Miss N. R. C. Davies for their helpful comments on this paper, and acknowledge the help and advise given by Miss D. Norris and the National Health Institute, who also supplied the controls for the toxigenicity tests.

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Group B Streptococcal Isolates During a Three-year Period

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Introduction

The grouping of beta-haemolytic streptococci by extracting the specific carbohydrate as described by Lancefield[8] is not a new technique. Commercial antisera are available to the 18 Lancefield groups so far recognised. These groups are designated alphabetically from A-T excluding I and J[3].

Group B streptococci *(Streptococcus agalactiae)* have been recognised as a cause of bovine infections for several years[11]. The isolation of *Str. agalactiae* from human sources has been reported with increasing frequency[1, 2, 4, 11, 12].

Group B beta-haemolytic streptococcal infection in neonates is now a well-recognised entity[1, 2, 6 · 12] and mother to baby transfer is suggested as the possible source of neonatal infections[4, 11].

The significance and frequency of group B streptococcal isolates from various sites in non-neonatal patients has not been widely reported. This study was set up to determine the frequency and importance of the isolation of these organisms from sources other than maternal and neonatal.

Materials and Methods

All clinical isolates of beta-haemolytic streptococci, which were resistant to 0.1 unit bacitracin or were sensitive but had a colonial morphology not typical to that of group A streptococcus, were included in the study which took place between 1 July 1971 and 30 June 1974. The organisms were obtained from patients in Christchurch, Christchurch Women's and The Princess Margaret Hospitals.

Lancefield grouping was carried out on all these organisms. The antigen was extracted by the heat extraction method of Rantz and Randall[9]. The gel diffusion method was used to group the antigen extract. Commercial antisera (Wellcome N.Z. Ltd.) was used throughout. Complete methodology may be found in a Technical Communication[13].

The following particulars were recorded. The name, age, sex, location in hospital, clinical history, site from which the organism was isolated for each patient as well as the amount of growth, other organisms present, haemolysis on human blood agar, bacitracin sensitivity (0.1 unit discs) growth in Bagg broth and the Lancefield group of each organism.

Results

The total number of non-group A streptococci isolated during the study period was 1095, 31 percent (338) were Lancefield group B. Sixty-eight percent (230) of isolates were from females, and 30 percent (102) from males, with sex not noted on 2 percent (6).

During the first year of the study 48 Lancefield group B streptococci were isolated (14

percent of the total non-group A streptococci), 112 (33 percent) group B beta-haemolytic streptococci in year two and 178 (53 percent) in year three. The number of organisms referred for Lancefield grouping has increased probably because of more awareness of the importance of differentiation of beta-haemolytic streptococci other than group A.

Group B beta-haemolytic streptococci were isolated from many different sites including the umbilicus of a 62-year-old male, the amniotic fluid and lungs of baby who died half an hour after birth. The lung isolate was obtained by post-mortem culture.

Three of the group B beta-haemolytic streptococci were isolated from both mother and baby. All of these isolates were in pure culture.

Case 1. Mother had group B streptococci isolated from her vagina and throat swab; and baby from swabs of both eyes, nose and umbilicus.

Case 2. Mother had group B streptococci isolated from her urine and amniotic fluid; and baby from a lung swab at post-mortem.

Case 3. Mother had group B streptococci isolated from her vagina; and baby from a stomach aspirate.

Twelve out of 33 neonates, had group B streptococci in pure culture (36 percent). All isolates were from pure, predominant or heavy growths.

Only one patient in the study was less than one year old and older than a neonate (up to four weeks of age). This child was two months old and the organism was isolated from a hip wound. The question to be considered, was this a neonatal induced infection? For simplicity this patient is included amongst the patients over one year of age.

Four out of 338 group B beta-haemolytic streptococci isolated were Bacitracin (0.1 unit) sensitive (1.2 percent). Male female ratio in patients over one year of age was 1 : 1.8 compared with 2.9 : 1 in the neonatal patients.

Discussion

The role of group B streptococci as a cause of neonatal infection has been well documented[1, 2, 6, 7, 11, 12]. The mother is generally accepted as the reservoir of infection. Vaginal carriage of these organisms is usually reported as between 5-10 percent[9]. Ruptured membranes predispose to the development of neonatal infection[2, 11] with group B beta-haemolytic streptococci. It is important that vaginal or cervical swabs from women with ruptured membranes are carefully examined for group B streptococci. The early diagnosis of a group B streptococcal infection in neonates enables early and appropriate antibiotic therapy to be instituted resulting in the early eradication of these organisms and hopefully a decrease in morbidity and mortality. It appears that penicillin G is the drug of choice[11], and a study is currently being prepared for publication on the antibiotic sensitivity of all Christchurch isolates of beta-haemolytic streptococci. Preliminary results confirm that penicillin G is the drug of choice.

The role of group B streptococci in human infections in general is poorly documented. The purpose of this study was to assess the distribution of group B streptococci in clinical specimens.

Isolates from the genital region accounted for 28 percent (94) of the total isolates (338). Were all these isolates commensal group B beta-haemolytic streptococci or were some of these organisms the cause of infection? The clinical histories were supplied with 36/89 of the vaginal and cervical swabs from which group B streptococci were isolated in pure or heavy culture. These included: abortion (complete, incomplete or threatened) at varying stages of gestation 10 (28 percent), puerperal pyrexia (post-ruptured membranes and forceps delivery) seven (19 percent), abdominal pain and/or vaginal discharge 10 (28 percent), and miscellaneous nine (25 percent) (prepubital infection, post-hysterectomy, pruritis vulvae, menorrhagia). The isolation of this organism in pure or heavy growth suggests it was the cause of an infectious process, probably endogenous in origin. Seventy-six (60/75) of the urinary isolates were in pure or predominant culture, with greater than 100,000 organisms per ml.

Fifty percent of all the group B streptococci, i.e., 169 were isolated in pure, heavy or predominant culture. There can be no dispute over the pathogenic role of group B streptococci isolated from CSF, blood cultures and aspirates. However, their pathogenic role in wound swabs is not as clear cut. Forty-one percent (31) were isolated in pure or heavy culture from wounds and may be presumed to have a significant role in the infectious process.

Table I. — Distribution of group B streptococci in patients over one year of age

Site	*Male*	*Female*	*Unknown*	*Total*	*% total isolates*	*% pure or pred. growths*
Genital	5	89	—	94	28%	53%
Urine	9	63	3	75	22%	76%
Wounds — general See Figure 2	39	36	1	76	23%	41%
Respiratory tract, Upper and lower	26	25	1	52	15%	45%
Blood cultures	2	4	—	6	2%	100%
CSF	—	1	1	2	<1%	100%

Table II. — Distribution of group B streptococci under heading Wounds — General in Figure 1

Site	*No*	*%*
Legs, feet, ankles, toes	27	36%
Finger and hands	8	11%
Hips	5	6%
Breast	3	4%
Aspirates (hip, knee fluid, ascitic fluid, amniotic fluid)	4	5%
Miscellaneous		
Wound tissue, bursae, scars, neck, forehead, umbilicus, sinus and wounds not otherwise specified	29	38%

Table III. — Distribution of group B streptococci in neonatal patients

Site	*Male*	*Female*	*Total*	*%*
Nose	6	5	11	33%
Eye	2	2	4	12%
Ear	2	1	3	9%
Umbilicus	4	1	5	16%
Rectum	1	1	2	6%
Groin	1	0	1	3%
Lungs (post-mortem)	3	1	4	12%
Stomach aspirate	—	1	1	3%
Endotracheal tube	2	—	2	6%
Total	21	12	33	100%

The increasing recognition and identification of these organisms indicates a need for awareness of the potential pathogenicity of these organisms. Braunstein *et al.*[2] found all of their non-genital isolates to be of possible clinical significance. The results of this current study also show that group B streptococci appear to have a pathogenic role in infection occurring in parts of the body other than the genito-urinary tract.

It is possible to sub-type these organisms but this was not attempted in this study. Reports[12] of sub-typing indicate that there may well be a varying degree of virulence between strains, and this may directly aid diagnosis and be of obvious epidemiological value when the source, spread and virulence of these organisms is investigated.

In conclusion the group B stretococci cannot in future be relegated to the role of "com-

mensal" as has happened in the past. Only by full investigation can the true pathogenicity of these organisms be assessed.

Summary

A total of 338 Lancefield group B beta-haemolytic streptococci were isolated between 1 July 1971 and 30 June 1974. Seventy-two percent of the isolates were non-genital. The distribution and relation to infection of these organisms is discussed.

Acknowledgments

I would like to thank Miss M. McIntosh and T. Schulze for their technical assistance and Mrs P. Goodger for clerical assistance.

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Lancefield Grouping of Beta-Haemolytic Streptococci

Over the past two years we have been Lancefield grouping our beta-haemolytic streptococci using a variation on the gel diffusion method of Lancaster[1]. The C-carbohydrate is extracted by the autoclave method of Rantz and Randall[2]. All beta-haemolytic streptococci isolated at Christchurch Hospital are Lancefield grouped.

We have found that increasing the size of one of the wells altered the antigen-antibody ratio and increased the sensitivity of the precipitin reaction. The increased sensitivity may also be due to different rates of absorption of the antiserum and the relatively watery antigen extract.

Increasing the size of the antiserum well to 3 mm (double the antigen well of 1.5 mm) appears to allow more antibody to diffuse on a wider front, hence the appearance of a longer and stronger line. We have been routinely testing our beta-haemolytic streptococci against antiserum to Lancefield groups A-K. Gel diffusion using 1 percent ion agar in 0.85 percent saline in a glass petri dish, with holes cut as shown in Figure 1 was used. The petri dish is incubated in a moist chamber at room temperature overnight and examined for precipitin lines.

The holes are cut in horizontal lines as we have access to a cutter which will cut two complete sets of holes at once, however the hole could be just as easily cut in the traditional circle, and performed on glass slides.

A comparison of the distribution of non-group A beta-haemolytic streptococci has been prepared for publication, and a comparison of all beta-haemolytic streptococci, their bacitracin sensitivity, grouping and distribution is currently being performed and will be submitted for publication shortly.

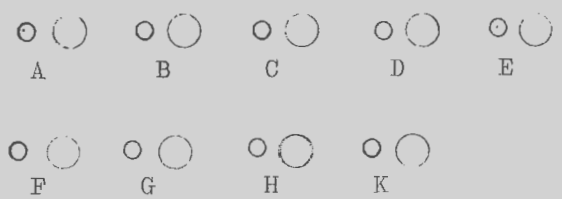

Figure 1. — Gel diffusion template. Antigen well diameter 1.5 mm; antibody well diameter 3.0 mm; well-to-well distance 4.0 mm.

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September 1975

The Haemolytic Anaemias—Part 2

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Unstable Haemoglobin Haemolytic Anaemias

In these anaemias the haemoglobin molecule present is unstable due to an amino acid substitution. The resultant anaemia varies in severity; splenomegaly is commonly found and urine pigmentation with a specific dipyrrole occurs. The blood film shows only a few irregularly shaped cells and microspherocytes; otherwise the film is normal. Heinz bodies may be seen in erythrocytes before splenectomy, but following splenectomy they are invariably found in high numbers. Heat incubation of blood of unsplenectomised patients results in the above inclusions[23].

Variable haemoglobin patterns[12, 36] may be found in this group of anaemias which show variable clinical results after splenectomy. Important is the precipitation of haemolysis by various oxidant drugs. Numerous diagnostic techniques are available[3] but studies to date have been unable to elucidate the relevance of Heinz bodies in this form of haemolytic anaemia[37].

The Thalassaemic Syndromes

This group of inherited disorders has a fundamental abnormality in that there is a defective rate of normal haemoglobin synthesis. Thalassaemia has a widespread distribution, particularly in those people of Mediterranean stock.

Classification

This is based on the abnormal haemoglobin pattern present. The α and β chains of haemoglobin are synthesised independently and are under separate genetic control. Hence there are two basic groups of thalassaemias; those relating to α chains (α thalassaemias) and those affecting β chains (β thalassaemias). In β thalassaemias Hb-A_2 or Hb-F is increased, there being an increase in δ chains of Hb-A_2 or γ chains of Hb-F. In α thalassaemia the levels of Hb-A, Hb-A_2 and Hb-F are depressed and an excess of β chains or γ chains occurs which aggregate to form Hb-H ($\beta 4$) or Hb-Barts ($\gamma 4$).

β Thalassaemias

(1) β Thalassaemia Major

This is the heterozygous form of the disease. Onset of this form of haemolytic anaemia occurs early in childhood, with pallor, hepatosplenomegaly and skeletal changes common. Growth retardation, periodic fever, cardiac enlargement, leg ulceration and cholelithiasis may also be found. Variants have been detected of thalassaemia major and the disease is progressive with haemolytic crisis rare, however. Death usually occurs from intercurrent infection, anaemia or cardiac failure.

Blood Picture

This resembles severe iron deficiency anaemia with marked anisocytosis, poikilocytosis and hypochromasia. Target cells are prominent. The MCV, MCH and MCHC are reduced but not in relation to the degree of anaemia. Erythroblastaemia is common as is punctate basophilia and polychromasia. A leucocytosis is common and platelets are found in normal numbers.

The osmotic fragility shows an increased resistance, and hyperbilirubinaemia may be seen. Electrophoresis demonstrates the haemoglobinopathy with marked increases in Hb-F. Bone marrow displays a very active erythroid hyperplasia.

(2) β Thalassaemia Minor

This represents the heterozygote state of the disease and may be symptomless or have only mild symptoms of anaemia. Splenomegaly may be present and cholelithiasis may occur. Anaemia may be precipitated by infection or pregnancy. Life expectancy is normal.

Blood Picture

This is similar to β thalassaemia major but to a lesser degree. A small amount of increase in Hb-F may be demonstrated in 50 percent of cases, but in most cases there is an increase in Hb-A_2.

(3) β Thalassaemia Intermedia

This disorder lies in severity between thalassaemia major and minor. Anaemia,

splenomegaly and bone changes are commonly found.

Diagnosis

Thalassaemia major presents no diagnostic difficulty after consideration of clinical, family and haematological findings. Thalassaemia minor requires to be distinguished from iron deficiency anaemia, and serum iron levels and quantitation of Hb-F and Hb-A_2 may be needed before a diagnosis may be firmly established.

Treatment

Supportive therapy by means of blood transfusion is the main form of therapy. Splenectomy may be beneficial in selected cases and vitamin supplement may be necessary if deficiency exists, particularly that of folic acid.

α Thalassaemias

In these disorders the deficiency of α chains results in a generalised depression of Hb-A, Hb-F, and Hb-A_2 with the appearance of either Hb-H ($\beta 4$) or Hb-Barts ($\gamma 4$).

α Thalassaemia Major

This represents the homozygous state and is incompatible with life. Examination of the blood of the hydropic infant shows a predominance of Hb-Barts.

α Thalassaemia Minor

This represents the heterozygous state and generally is symptomless. The MCV may be slightly reduced and there is a slight increase in osmotic fragility.

Hb-H Disease

This is a combination of heterozygous α thalassaemia with an unknown gene, resulting in minor variable blood picture changes. Hb-H may be demonstrated by electrophoresis and denatured Hb-H may be shown by supravital staining techniques.

Acquired Haemolytic Anaemias

This grouping of haemolytic anaemias is based on the presence of an acquired extracorpuscular haemolytic mechanism. Autoantibodies may be demonstrated in many cases, but in most the mechanism of haemolysis is unknown. This form of haemolytic anaemia is the commonest found in adults and varies greatly in severity. Acquired haemolytic anaemia may be subdivided:—

(a) According to the clinical course into acute or chronic.

(b) According to aetiology into primary (idiopathic) or secondary (symptomatic).

Acute acquired haemolytic anaemia occurs most commonly in children, runs a short course and invariably is of unknown aetiology. Chronic acquired haemolytic anaemia is more common in adults, persists for many months, and usually has a demonstrable antibody present. Chronic cases are more common than the acute.

Clinical Features

(1) Acute Acquired Haemolytic Anaemia

The onset of this condition is rapid with jaundice, fever, abdominal pain and vomiting common findings. Haemoglobinuria is usual and oliguria may occur. A palpable spleen may be found. Death may result from renal failure but this is rare. Haemolysis ceases spontaneously after weeks or months and is followed by recovery. This acute form of haemolytic anaemia may be associated with a prior upper respiratory tract infection, and it may complicate virus pneumonia or infectious mononucleosis, where specific[2, 24, 28, 34] or nonspecific blood group autoantibodies may be demonstrated.

(2) Chronic Acquired Haemolytic Anaemia

This is the commonest form of haemolytic anaemia of adults. The idiopathic form is found most commonly in females and individuals over 50 years of age. The symptomatic form may complicate various diseases, for example lymphomas[4, 29], certain leukaemias and disseminated lupus erythematosis, or may be associated with various drugs, for example methyldopa[9, 30]. The onset is insidious with symptoms of anaemia. Haemolytic anaemia may be the initial symptom of an underlying disease process. Splenomegaly is common, and hepatomegaly may also occur. Lymphadenopathy may be seen in symptomatic cases, as may purpura.

Blood Picture

Typical findings include an anaemia with reticulocytosis, spherocytosis, bilirubinaemia, a raised sedimentation rate and a positive direct Coomb's test. Spherocytosis is accompanied with increased osmotic fragility but not as marked as in hereditary spherocytosis. During a haemolytic phase a leucocytosis is common, and a lowering of the platelet count may be found.

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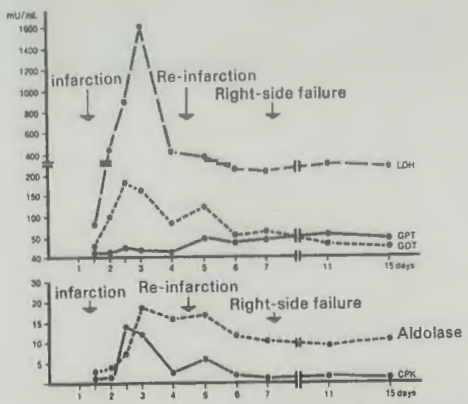

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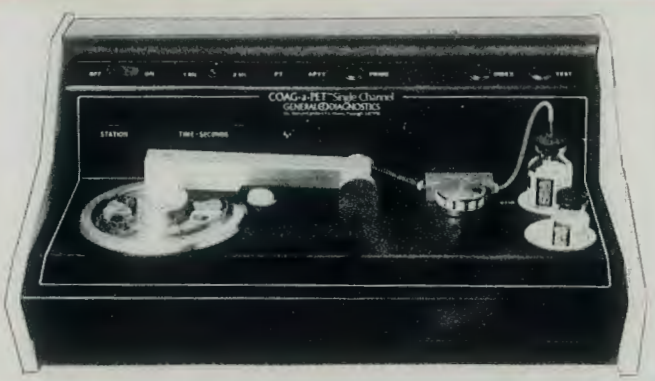

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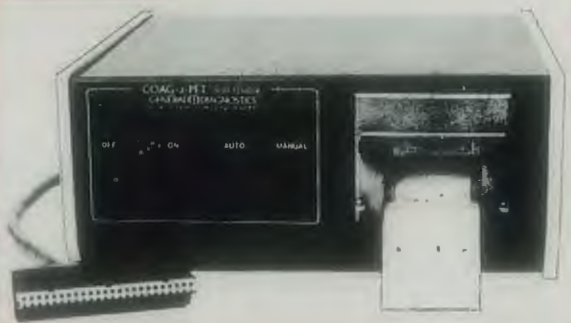

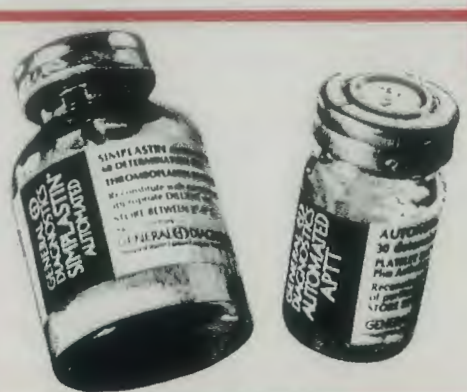

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APPARATUS FOR SALE

Tasman Vaccine Laboratory has amalgamated a number of previously separated laboratories, and has purchased new equipment for some areas, resulting in a surplus of used, but good apparatus. The following list includes some of the many items available at very reasonable cost.

	Purchased
Ovens, MLS brand and Contherm brand. Various sizes.	1973 & 1974
Sartorius Top-pan balance. 1000 g x 0.005 g	1974
Stanton Analytical Balance. 200 g x 0.0001 g	1971
Water bath, Grant SB15, 0-100 degrees C, 0.2 degrees C, with two sets of test tube racks	1972
pH Meters. Several models, mostly Radiometer brand	—
Autotitrator, Jones Model 17, Type 2. Input 1-100 microamps, 1500 ohms. Output 6V 3 amp. with burettes and solenoid valves	—
Fraction Collector, LKB 7000 Ultra Rac, and Flow Stopper Valve	1972
U/V Absorptiometer. LKB 8300 Uvicord II	1972
Fraction Collector, Isco Golden Retriever Pup Model 1200, with 95 tubes, 19 racks, rack stand, and photocell drop detector	1974
Fraction Collector, Gallinkamp Model 291, with syphon balance and circular collector plate	1970
Refractometer, Bellingham and Stanley, Abbe. R.I. range 1.3000 to 1.7000.	1974
Autoclave, Mercer electric. 2.3kw, 230 v. Stainless steel; hinged lid	1974
Peristaltic Pump, Quickfit 14 channel, Type 10 PP60, variable speed, forward and reverse	1970
Isomantle, 4 unit, each 5in diameter, each controlled by simmerstat	—
Isomantle, MUL 500, 160 watt 4½in diameter	—
Isomantle, MUL 3C, 2 heat—2 x 250 watt 7in diameter	—

For prices or details please contact Mr L. Stonyer, phone Upper Hutt 87-139, Telex, NZ 31265.

Autoantibodies may be demonstrated *in vitro* in many cases of acquired haemolytic anaemia and these may be differentiated on the basis of blood group specificity, especially the Rhesus blood group system, or on the immunoglobulin type found[7]. Those antibodies found secondary to viral illness and Raynard's syndrome are predominantly of the cold type. An animal model of this form of haemolytic anaemia has been demonstrated in mice[16].

Diagnosis

This is based on the typical findings of a haemolytic anaemia, plus a positive test for the presence of antibody, although as noted the presence of antibody may be a transient finding.

Course and Prognosis

The course of the disease in a particular patient is difficult to predict as haemolysis may occur very rapidly without warning. In idiopathic cases prognosis, however, is generally better in the cold than warm antibody type. High amounts of autoantibody production and a low platelet count are poor signs. In symptomatic cases prognosis depends largely on the underlying disorder.

Treatment

Basic supportive therapy is blood transfusion and adrenocortical steroid hormones. Splenectomy may be of help in selected hormone resistant cases. Failing this, immunosuppressives may be used[38].

Haemolytic Anaemias Associated with Haemoglobinuria

Normally haemoglobin is broken down within the cells of the reticuloendothelial system and the level of free haemoglobin in plasma is small. However, when intravascular haemolysis occurs and the plasma haemoglobin exceeds the renal threshold haemoglobinuria results. It is important to differentiate this from haematuria.

Associated with intravascular haemolysis may be a variety of symptoms, including rigors, headache and malaise. In the acute condition acute tubular necrosis may lead to acute renal failure, characterised by oliguria (or anuria) and uraemia. This is most commonly seen after an incompatible blood transfusion. Methaemoglobin may be present in plasma. This is a haematin-albumin complex found classically in intravascular haemolysis. Haemosiderin derived from haemoglobin breakdown may be excreted and is readily demonstrated by Perl's test.

Paroxysmal Nocturnal Haemoglobinuria (PNH)

This rare condition is a chronic haemolytic anaemia with associated intermittent haemoglobinuria and persistent haemosiderinuria. Basically there is an acquired defect of the red cell membrane which is readily lysed by complement of normal serum.

Clinical Features

PNH is most commonly found in adult life and is not hereditary. The onset is insidious and haemoglobinuria during or following sleep is classically found. Lower abdominal pain, hepatosplenomegaly and venous thrombosis may be seen. Attacks may be precipitated by various factors including infection and administration of drugs.

Blood Picture

Macrocytosis, polychromasia, reticulocytosis, neutropenia and mild thrombocytopenia are commonly found. The osmotic fragility is normal. Hyperbilirubinaemia may be found and occasionally the Coomb's test may be positive.

Diagnosis

This is confirmed by Ham's acid-serum test, where erythrocytes from a case of PNH readily lyse in acidified normal serum.

Course and Prognosis

This chronic disease varies in severity. Spontaneous cure may occur but invariably death occurs because of complication from anaemia, infection or thrombosis.

Treatment

This is unsatisfactory. Symptomatic treatment by blood transfusion and avoidance of precipitating factors are the basis of treatment. Steroids may be of help in a few cases; splenectomy is contraindicated.

Paroxysmal Cold Haemoglobinuria

This rare condition follows exposure to cold which may be local or generalised. Most cases are found related to syphillis, with exposure to cold resulting in abdominal pains, cramps, rigors and pyrexia. Haemoglobinuria follows an attack; splenomegaly may be found and urticaria may result.

Diagnosis

This is achieved by finding a cold haemolysin in the patient's serum by the Donath Land-

steiner test.

Treatment

This is prophylactic, avoidance of cold wherever possible. Therapy for underlying syphillis is required and steroid therapy may be useful in severe cases.

March Haemoglobinuria

This rare disorder occurs after exercise, with symptoms of haemoglobinuria, with on occasions nausea and variable abdominal or lower limb pain. Anaemia is rare. The cause of march haemoglobinuria is thought to be intravascular haemolysis due to the trauma on the blood within the vessels of the soles of the feet.

Haemolytic Anaemia Due to Drugs and Chemicals

Two basic groupings are found in cases of haemolytic anaemia of this type. The chemical may cause haemolysis by one of two actions:—

(1) By a direct toxic action on the erythrocyte. In these cases haemolysis regularly follows ingestion of the chemical and the degree of haemolysis approximates to the dosage taken.

(2) The chemical may cause haemolysis because of idiosyncrasy or by inducing the production of antibody which may or may not react with the patient's erythrocytes depending on whether the chemical is present.

Idiosyncrasy to a particular drug or chemical is probably the most common mechanism causing haemolytic anaemia within this group and in many cases relates to an enzymatic deficiency, for example G6PD. Anaemia in these cases is acute, severe and haemoglobinuria is common. Death may ensue due to acute anaemia, shock or acute renal failure. Drugs causing autoimmune haemolytic anaemia are extremely common[1, 26, 39].

Blood Picture

The degree of anaemia is variable and is accompanied by findings of direct toxic action on the erythrocyte, namely fragmentation, irregular forms, spherocytosis and basophilic stippling. Various aromatic nitro and amino compounds cause the formation of Heinz bodies and may also produce methaemoglobinaemia and sulphaemoglobinaemia. A negative Coomb's test is usually found, as is a neutrophil leucocytosis and thrombocytosis.

Haemolytic Anaemia Associated with G6PD

Various enzymatic deficiencies have been found within erythrocytes; of note is that due to G6PD which has a high incidence in Negroes but may be found in high incidence in various other races. Genetic transmission is by intermediate dominance of a sex-linked gene. The trait is fully expressed in the hemizygous male and in homozygous females who have been shown to have a double population of erythrocytes; one population with normal enzymatic activity and one with a marked deficiency.

Clinical Features

These are those of an acute haemolytic anaemia. Generally the dose of drug ingested relates to the degree of drug-induced haemolysis. Haemoglobinuria is variable in severity and abdominal and back pain may occur.

Various drugs have been noted to cause haemolysis in G6PD subjects, and include antimalarials, sulphonamides, sulphones, analgesics and water soluble vitamin K analogues.

Blood Picture

Unexposed individuals have a normal blood picture except for decreased levels of glucose-6-phosphate. In a haemolytic phase Heinz bodies, basophilic stippling, polychromasia and anisocytosis may be found. Osmotic and mechanical fragility studies are normal.

Diagnosis

G6PD should be suspected in any case of acute haemolytic anaemia where the Coomb's test is negative, and particularly if the patient is of Negro, Mediterranean or Oriental stock. In suspected cases glucose-6-phosphate should be measured directly.

Favism

Favism occurs is individuals sensitive to the fava bean, *Vicia faba,* either by inhalation of pollen of the plant or by ingestion. Haemolysis may be acute if pollen is inhaled or delayed up to six hours after ingestion of the bean. Anaemia may be severe and recovery is invariably spontaneous.

Favism occurs mainly in individuals of Mediterranean stock, or in those other people with G6PD. The presence of G6PD does not predispose an individual to favism; because of this fact it is thought that hypersensitivity is important in precipitating an attack.

Lead Poisoning

Exposure to lead results in basophilic

stippling of erythrocytes, haemolytic anaemia, polychromasia and reduced osmotic fragility. Haemolysis of erythrocytes plays a secondary role to marrow suppression. In the past basophilic stippling has been a commonly used method of screening those in contact with lead as evidence of lead poisoning. However, it is now realised that the degree of stippling does not parallel the level of poisoning.

Haemolytic Anaemia Associated with Infections

Frank haemolytic anaemia may result following infection, particularly to *Clostridium perfringens, Bartonella bacilliformis,* and the malarial parasite. Haemolysis in cases of this type may be severe with features of intravascular haemolysis.

Microangiopathic Haemolytic Anaemia

In this form of haemolytic anaemia a characteristic blood film is seen with numerous distorted, small, fragmented erythrocytes. The erythrocyte deformity is thought to result from small vessel narrowing. Most cases are found in those individuals with severe renal disease associated with arteriolar and capillary thrombosis, with or without necrotising arteritis.

Haemolytic-Uraemic Syndrome of Infancy

This form of haemolytic anaemia follows a viral infection generally in the first year of life. Pyrexia, vomiting and diarrhoea are common, followed by intravascular haemolysis. The prognosis is poor: treatment consists of that for renal failure plus transfusion. Steroids have been used with some success, indicating the role of immunity in this condition.

Cardiac Haemolytic Anaemia

Haemolytic anaemia following open heart surgery, particularly in cases of valve prosthesis, may be mild or massive. Mechanical trauma is the cause of haemolysis in many cases and it follows that the symptoms and signs of intravascular haemolysis result. Autoimmunity is thought to play a part in those cases where direct trauma to the erythrocyte cannot be demonstrated[13, 22].

Disseminated Intravascular Coagulopathy (DIC)

Associated with the syndrome of DIC is erythrocyte fragmentation which directly relates to the degree of defibrination[19]. It is thought that the erythrocyte deformities are caused by direct trauma in small vessels, and result in a haemolytic anaemia which in some cases may be severe.

Haemolytic Disease of the Newborn

This complex, well-documented condition is due to antibody produced by a mother, in response to a previous stimulus, crossing the placenta and interacting with the appropriate antigen (if present) on the surface of foetal erythrocytes. Various blood group systems have been implicated as causative agents in this condition, the more important being the Rhesus, ABO and Kell blood group systems.

Clinical Features

These may vary from a mild asymptomatic jaundice or anaemia to death *in utero* depending on the degree of haemolysis occurring. Important features include the development of hyperbilirubinaemia and subsequent kernicterus if severe and untreated.

Blood Picture

Studies on cord blood show an anaemia, erythroblastaemia, reticulocytosis, leucocytosis, a normal or lowered platelet count, and a varied osmotic fragility depending upon the degree of spherocytosis present which relates to the blood group system involved[33]. The Coomb's test may be positive in most cases, and modifications of this test have been used to demonstrate previously undetectable antibody.

Treatment

This consists of exchange transfusion and UV light therapy. Of importance is prophylaxis by the use of post-delivery injection of the mother with antibody and agents such as barbiturates.

In summary haemolytic anaemias form a broad complex unresolved group of haematological disorders with a most varied and interesting presentation. A knowledge of the basic groupings of this disease is important, so that recognition of the condition and appropriate treatment may be given.

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Technical Communications

A Case of Congenital "Acute Myeloblastic Leukaemia" in Down's Syndrome

Described at the Annual Conference, Palmerston North 1975

Baby McG. was born on 1 March 1975 after 42 weeks gestation with a birth weight of 3,125gm. He showed the clinical features of Down's Syndrome (mongolism). Cytogenetic studies on peripheral blood lymphocytes confirmed this.

The mother was a normal-looking 24-year-old European who had previously given birth to a normal child after toxaemia in the 39th week. This pregnancy was uneventful except for breech presentation diagnosed two weeks antenatally by abdominal x-ray.

The results of the cord blood were as follows: Hb 17.4mg/100ml, PCV 59 percent, MCHC 30 percent, Group B D positive, immune anti-B screen negative, direct Coombs negative, total bilirubin 1.2mg/100ml. A blood film was not done. At three days the baby developed jaundice. Bilirubin was 13.4mg/100ml, this fell to 8.0mg/100ml on the sixth day. On the seventh day the total bilirubin was 10.6mg/100ml, direct bilirubin 7.1mg/100ml. A blood screen showed Hb 17.4mg/100ml, PCV 56 percent, MCHC 31 percent, WBC 60,000, polymorphonuclear neutrophils 21 percent, lymphocytes 25 percent, blasts 54 percent, platelets 195,000, retics 3.2 percent.

A physical examination showed an enlarged liver, the lower edge some 4cm below the rib cage, a palpable spleen, and a heart murmur. On the 12th day it was noted the baby's fontanelle was tense and he had head retraction.

The CSF was mildly blood stained. The supernatant contained free haemoglobin. There were 1,400 cells/cmm with 190 leucocytes/cmm: 90 percent of these were polymorphs. A gram stain showed a few polymorphs but no organisms. There was no growth after 48 hours incubation. The glucose was 55mg/100ml and the protein was 260mg/100ml.

Bone marrow was aspirated with difficulty from the iliac crest; microscopically the fragments were grossly hypercellular. Polymorphonuclear neutrophils 40 percent, metamyelocytes 3 percent, myelocytes 1 percent, blasts 44 percent, lymphocytes 12 percent. There was a predominance of red cell precursors with some showing megaloblastic features. A periodic Acid Schiff stain showed variable positivity in the nucleated red cells.

Over the next two weeks there was a gradual change in the haematological condition of the patient. On 26 March Hb 10.0mg/100ml, PCV 35 percent, MCHC 29 percent, WBC 18,000, polymorphs 15 percent, lymphocytes 45 percent, monocytes 2 percent, eosinophils 11 percent, basophils 8 percent, blasts 19 percent, platelets 83,000. Meanwhile, he developed pneumonia which was treated with ampicillin and penicillin. An x-ray at this stage showed abnormal pulmonary shadows and an enlarged heart shadow. A later x-ray was consistent with a left to right chamber shunt within the heart.

He was discharged on 26 March with no medication, to be seen as an outpatient in three weeks' time. On 14 April a blood test was done. Hb 7.7mgm/100ml, PCV 24 percent, MCHC 32 percent, WBC 9,400, with 2 percent blasts, polymorphs 36 percent, lymphocytes 53 percent, monocytes 7 percent, myeloctyes 2 percent. He was admitted on 17 April for transfusion and for failure to gain weight.

Following transfusion of 100ml of blood the Hb was 11.3mg/100ml, PCV 35 percent, MCHC 32 percent, WBC 11,800, blasts nil. Other diff. as on 14 April. He developed pneumonia which improved with antibiotics. He then developed congestive heart failure which was treated with digoxin. He steadily deteriorated and died on 3 May of heart failure. Bone marrow aspiration showed that the marrow was cellular with approximately 70 percent myelocytes, 10 percent promyelocytes; many of the nucleated red cells showed lobulation. Consent for full post-mortem examination was not given.

There is divided opinion in the literature, whether this phenomenon is a leukaemoid reaction or in fact acute leukaemia, in which there is likely to be a high incidence of spontaneous remission.

A good account is given in Oski and Naiman's book "Haematological Problems in the Newborn" and also in Carl H. Smith's "Blood Diseases of Infancy and Childhood".

Conclusion

In this child there was strong haematological and clinical evidence of acute leukaemia. He had blasts in the peripheral blood and bone marrow and possible infiltration of the liver, spleen and meninges.

Acknowledgments

I would like to thank Dr D. Beasley for permission to publish this paper, Mr K. B. Ronald for the opportunity to present it, and Mr J. Marr for his constructive criticism.

I would be very interested to have details of similar cases found in other laboratories.

L. C. Dent, ANZIMLT,
Public Hospital,
Whangarei.

October, 1975.

A System for Stock Records

It is thought that perhaps a short explanation of a card system of stock recording, which is used by the pathology services of the North Canterbury Hospital Board for all the Board's laboratories, may be of interest to some other laboratories.

About seven years ago the old Stock Book system of recording stock purchases and costs was discarded. The reasons for this were that it did not give all the details required, was tedious to fill in properly and consequently, at times, was impossible to understand by anyone other than the sole user.

After some trials, a "Stock Card" of precise design was printed and put into use in 1969. It has proved to be most satisfactory and has not been altered since its first printing. The

only alteration we would make in the next printing is to have the Board's title, which is on one side only, printed at the bottom, as on the "Stores Issues" card to be described later.

The card is simple for anyone to write up and to read and gives all details, except stock level, required to be known about an article for re-ordering, etc. The present stock in hand is shown on the Stores Issues card.

"Shunic", style "QQ" signals are useful to clip on to cards with items on order, or awaiting payment, etc.

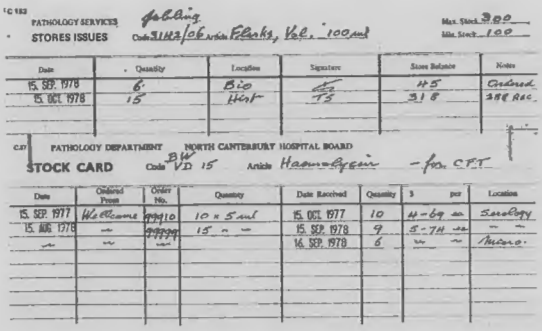
The Stores Issues card is of similar set-out with suitably spaced and labelled columns and this was introduced a year ago.

The "Store Balance" can be entered on a theoretical basis on issue of items and physically checked from time to time and used as a stocktaking record. So far this card has proved very satisfactory.

Having two sets of columns on a card was considered, but this seemed too cramped for easy use, so that the whole width of the card was used for clarity.

Both cards are standard 8in × 5in in white index board, 165gsm, with RED print and lines for the Stock Card and GREEN for the Stores Issues card which immediately avoids confusion. These colours allow entries in blue or black handwriting to stand out prominently. Both cards are printed in tumble fashion on both sides.

The figures show one face of each card, partly filled in, as an example of how it may be used.



PATHOLOGY SERVICES Jubilee
STORES ISSUES Code 3142/06 Article Flasks, Vol. 100ml
Max. Stock 300
Min. Stock 100

Date	Quantity	Location	Signature	Store Balance	Notes
15. SEP. 1978	6	Bio Hist	TS	45	Ordered 300 Rec.
15. OCT. 1978	15			318	

PATHOLOGY DEPARTMENT NORTH CANTERBURY HOSPITAL BOARD
STOCK CARD Code VD 15 Article Haemolysin - for CFT

Date	Ordered From	Order No.	Quantity	Date Received	Quantity	$ per	Location
15. SEP. 1977	Wellcome	99410	10 × 5ml	15. OCT. 1977	10	4-69 ea	Serology
15. AUG. 1978	"	99999	15 " "	15. SEP. 1978	9	5-74 "	" "
"	"	"		16. SEP. 1978	6	" "	Micro.

Cards can be filed in standard filing cabinets or, better probably, in a "Rotascan" file. It has been found desirable to divide into sections, such as "general", "chemicals", "glassware", "serology", etc.

We have about 3,000 or so Stock Cards which are continually being added to and Stores Issues cards for most of those items kept in store, but not for items (such as serology reagents) issued directly to laboratories where individual records are kept.

Summary

A card system for recording purchases of all laboratory articles and for issuing expendibles is described. This may draw forth some constructive comments or criticisms.

D. H. Adamson, FNZIMLT,
Chief Technologist,
Pathology Services NCHB,
Christchurch Hospital.

September, 1975

Order Form

Laboratory ware and Chemicals

SPECIAL OFFER means big savings for you!

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Please telephone us if you have any questions, or if you require other items not listed.

MEDICAL TECHNOLOGY	Cat. No.	Price $	Quantity
Pasteur Pipettes – Clay Adams 5¼"	4641	6.00/2½ gr.	
Pasteur Pipettes – Clay Adams 9"	4645	7.00/2½ gr.	
Disposable Serological Pipettes:			
1 ml, grad. 0.01 ml, non-sterile. Volac	RTM804	8.00/100	
1 ml, grad. 0.1 ml, non-sterile. Volac	RTM805	8.00/100	
1 ml, grad. 0.01 ml, sterile and plugged. Volac	RTM804/PS	9.00/100	
1 ml, grad. 0.01 ml, sterile and plugged Volac	RTM805/PS	9.00/1000	
Disposable Test Tubes, 50 x 7 mm. Durham. Volac	L1451	14.00/1000	
Disposable Test Tubes, 63 x 9.5 mm. Durham. Volac	L1452	15.00/1000	
Hydrochloric Acid AR. 2½ lit. BDH	10125	8.70	
Sulphuric Acid AR. 2½ lit. BDH	10276	8.70	
Acetic Acid AR. 5 pt. Mallinckrodt	2504	9.90	
Di Ethyl Ether, Anhydrous AR. 5 pt. Mallinckrodt	0848	12.70	
Toluene AR. 8 pt. Mallinckrodt	8608	16.50	
Xylenes AR. 8 pt. Mallinckrodt	8668	16.00	
Methanol AR (permit required) 5 gal. Mallinckrodt	3016	25.00	
Pipette Controller, 2 20 ml. Volac	RTM820	13.00	
Pipette Controller, 0.1-2 ml. Volac	RTM821	13.00	
Disposable Centrifuge Tubes, 10 ml, graduated polypropylene, cap attached	10CT	32.00/1000	
Eppendorf Autopette Tips 0-100 ml size		12.00/1000	
Fibrotips		18.00/1000	
Fibrocups		8.00/1000	
Protosol – Laboratory Cleanser, 10 lb		10.00	
Protosol – Laboratory Cleanser, 25 kilo		45.00	

TEAR ME OFF AND POST ME NOW!

Please charge

Address details

Delivery details

Order No.

SB 11981

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Introducing the IL513.

The blood gas analyzer that leaves nothing to chance.

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   A pushbutton at right of panel provides 10x expansion of Po_2 readout for patients on 100% oxygen.
2. **Calculated Values** — Derived results for Base Excess, Actual Bicarbonate and CO_2 Content displayed when the Calculate Button is pressed. Time for complete analysis: 72 seconds.
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4. **Fingertip Control** — All command console buttons and adjustment controls are on the front panel, within easy reach of the operator. Calibration Buttons 1 and 2, used in sequence, introduce high buffer and low gas simultaneously for balance calibration, then low buffer and high gas for slope calibration. Each step is preceded by automatic flushing to remove contaminants. Slope calibration is usually not required more than once each lab shift. Sensitive, fingertip control for microsampling is on right of panel, alongside aspirator and directly under measuring tank for precise visual monitoring of microsample.
5. **No Sample-Splitting** — Aspirator needle automatically draws a single 500μl sample when Sample Button is pressed. When button flashes and operator withdraws syringe, the needle retracts automatically into flushing chamber for thorough cleansing.
6. **Pre-Heater** — Blood sample is aspirated directly to this unit, which heats sample (including iced blood) to 37°C rapidly. Result: lengthy equilibration times are eliminated.
7. **Visible Bubble Chambers** — Transparent, and located on front of instrument, allowing operator to confirm visually that Lo or Hi gas is flowing, and at the proper rate. Purge button atop each chamber evacuates room air to help speed calibration and assure equilibration accuracy.
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9. **Nothing To Hide** — See-through plumbing shows how user-oriented design allows operator to identify a malfunction immediately and correct problem quickly. Every tube, tank and electrode is transparent, accessible, repairable or replaceable in minutes. These are bottles for automatic introduction of buffers and flush solution, and for the collection of waste solution. Buffer pouring chores for each analysis, along with buffer contamination problems, have been eliminated.
10. **Hard Copy** — Optional IL315 Ticket Printer provides measured and calculated values on individual cards in laboratory format. Automatic printing of 3 copies eliminates transcribing errors.

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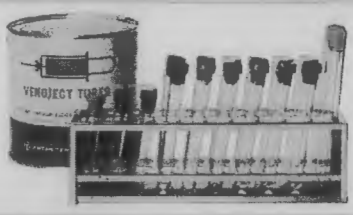

* Blood Testing System.
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Less blood
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Introducing the

B-D UNOPETTE Disposable Diluting Pipette

a complete micro-collecting dilution and reagent system, for blood samples in the Clinical Laboratory.

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A wide range of UNOPETTE tests are available

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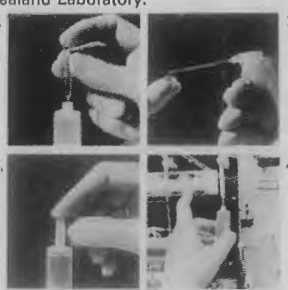
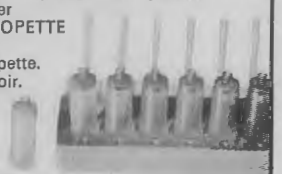
"A laboratory test is no better than the specimen and the specimen no better than the manner in which it was collected."

BD UNOPETTES are manufactured to exacting standards for the New Zealand Laboratory.



Instructions: 1 Puncture reservoir diaphragm with pointed polystyrene shield. 2. Make finger puncture. Allow blood to fill UNOPETTE capillary pipette. Carefully wipe excess blood from outside of pipette. 3. Seat pipette securely in reservoir. Gently squeeze reservoir several times to rinse all blood from pipette. Mix by inversion. 4. Insert aspirator tubing for pre-diluted samples in reservoir.



SB 11982

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Correspondence

"He's got 'em on the list:
And they'll none of 'em be missed"
(The Mikado)

Sir, — The present change in the units for reporting laboratory results to clinicians offers an opportunity to improve our communication in one important but neglected respect. This is the implied precision of the results we report.

How many of us have for years reported results (units usually omitted) such as "urea 38" or "glucose 212" or "bilirubin 10.2" or . . . without considering the apparent confidence in our results which we have been conveying to our customer, the clinician treating the patient? Now, in fact, we would not be so naive as to believe that we could consistently distinguish a sample with a glucose content of 210 mg/dl from one with 214 mg/dl: yet we have persisted in implying that we can confidently assure the recipient of our report that the glucose content is between 211.5 and 212.5 mg/dl, or at least that the result is 212 with a standard error of ±0.5 mg/dl. The present writers have become acutely aware of this problem as a result of the lipid survey which we have been conducting. From this we have found that there is no laboratory in this country which is justified in quoting either cholesterol or triglyceride results to better than the nearest 10 mg/dl: only a small minority, in fact, are justified in quoting results to within ±20 mg/dl for cholesterol or ±50 mg/dl for triglycerides (between batch precision). Despite this, the few laboratories that have adopted mmol/litre for lipid results are at present quoting their results to a second decimal place, which implies a precision actually greater than claimed previously.

In some countries it is mandatory for analytical results to be quoted with confidence limits. This procedure is certainly worth considering as an aid for those who must interpret our results. Many, however, will probably consider that quoted confidence limits lead to as many problems of interpretation as the raw results. This is arguable; but it behoves us all to think about the relationship between precision which we are achieving and which we imply in our reports. We should look at our quality control charts and survey performances and ask ourselves how many significant figures we are justified in reporting. The change of unit system provides a most convenient opportunity to drastically reduce the number of "significant" figures which we report: and they never will be missed.

M. Lever,
C. M. Andre,
T. A. Walmsley,
D. J. Munster,
Clinical Biochemistry Department,
Christchurch Hospital.

January, 1976

Another way to make results meaningful is to quote the current standard deviation obtained with the method, on the report. This provides the most precise information available for the purpose of interpreting the report. Some laboratories are doing this and this could be regarded as providing confidence limits. Although percentage standard deviation would be the obvious way to express this we give the actual figures obtained, with concentrations of substances rather higher than normal. The idea is that this figure can be immediately applied without the obstacle or inhibition of arithmetic and the justification is that in our experience the amount represented by the standard deviation does not vary greatly with varying levels of concentration. Some part of it will be due to irreducible calibration limitations and consequently different levels would and do require different percentage standard deviations. Such an addition to the report is complementary to the excellent point made in this letter which is illustrated by a communication in No. 6 of the NZACB News, 1975, reproduced here by courtesy of that publication. — The Editor.

INTERPRETATION OF BLOOD LIPID ANALYSES

We have asked a small number of clinicians and senior medical students what sort of uncertainty they expect in the cholesterol and triglyceride results that they obtain from the laboratory. Some had never thought about it, and one medical student was rather worried because his blood cholesterol appeared to have increased by 10 mg/dl during one year. Others

suggested that they would expect 95 percent confidence units to be ±5 to 10 mg/dl. and only one or two expected that results could be more than ±20 at 260 mg/dl.

For comparison, we estimated from the results of our current lipid survey the number of laboratories which are achieving various levels of precision. We calculated the coefficient of non-determination which correspond to the 95 percent confidence intervals in the Table below, and it will be seen that only a minority of laboratories are meeting the commonly expected standards of precision for cholesterol analyses. In the case of triglycerides, most laboratories have 95 percent confidence limits which are greater than the text-book normal range.

In the Table, we have imagined that a clinician is presented with a result giving cholesterol as 260 mg/dl and triglyceride as 150 mg/dl. The quoted limits are those within which the true cholesterol and triglyceride levels would fall 19 times out of 20. Fifty-three laboratories report cholesterol results, and 41 triglycerides, so that there are more than 10 laboratories which are not achieving any of the quoted levels of precision.

Cholesterol Limits	No. of Labs	Triglyceride Limits	No. of Labs
(mg/dl)		(mg/dl)	
250 - 270	1	130 - 170	1
240 - 280	10	100 - 200	7
220 - 300	36	50 - 250	31

Clearly there is a need for the clinician to be more aware of the limitations of the laboratory data presented to him, and in this context it is interesting to note that two independent studies (reported in 1968 and 1970) have estimated the minimum required precision for cholesterol analyses as ±40 and ±34 (95 percent confidence limits). If this is still an acceptable standard for diagnostic applications, a majority of New Zealand laboratories is achieving it. There is also a need for laboratories to be more aware of the limitation of their own results, and reporting such cholesterol results as "243" or triglycerides as "182" implies a level of precision which not one is achieving. With the change to SI units it is hoped that these third significant figures will be dropped, which will go a small way to remove the communication gap highlighted in this item.

D. J. Munster and M. Lever,
Clinical Biochemistry Department,
Christchurch Hospital.

Book Reviews

Review of Physiological Chemistry. Harold A. Harper, 1975. 15th Edition. 570 pages. illustrated. Published by Lange Medical Publications. Price $NZ11.50. N. M. Peryer Ltd., Christchurch.

First published in 1939, this is the 15th edition. As usual the editors have paid attention to recent discoveries and several sections have been added to or partially rewritten. Both in terms of nomenclature and structural formulae, the book has been updated throughout. It is disappointing in view of current trends to find that there is no mention of SI Units in quoting normal values in the chapter on blood.

Material on gas-liquid chromatography has been increased and a small section on thin layer chromatography included. The section on porphyrias now includes the results of recent studies on the activity of amlev synthetase and its role in the production of porphyria. The results of research on the administration of vitamins has also been included and the chemistry of lipoic acid now shows decarboxylation pathways.

In the chapter on enzymes a section on metal ions has been inserted and existing material expanded and updated. Plasma protein now includes sections on the structure of immunoglobulins and a discussion of multiple myelomata.

The chapter on metabolism and carbohydrates has been revised and some of the metabolic pathways modified. A rearrangement in sequence of topics makes the chapter easier to follow.

Several additions have been made to the sections on catabolism of amino acids and there is a much longer section on the biosynthesis on non-essential amino acids, chiefly glutamine

and glutamate. Mention is made of the newer automated techniques for large-scale screening of amino acids and the detection of metabolic defects. Liver function tests remain largely unaltered but some obsolete tests and the section on detoxification have been deleted.

In the section on hormones the new nomenclature of I_3Thn and Iu Thn replace T_3 and T_4. There is less material on laboratory diagnosis and treatment.

The section on diabetic states has been rewritten and now includes abnormal metabolism of the liver during diabetic instability. The chemistry of the adrenal medulla has been rewritten and tests of adrenocortical function have been tabulated for easy reference. There is an additional large section on the gastrointestinal hormones in this volume.

Colorimetry has a section on specific dynamic action plus a rather interesting energy equivalents chart but the comments on parenteral nutrition have been removed.

The appendix of abbreviations and alternative terminology has been correspondingly revised.

The success and popularity of this review is due to a great extent to the efforts made to keep it abreast of current developments in physiological chemistry and there has been continuous revision by the author and his collaborators over the years.

This is an invaluable textbook for those requiring an introduction to the subject and for those preparing for examinations in the medical laboratory sciences.

Janice Parker.

Spores VI: Selected Papers from the Sixth International Spore Conference, Michigan State University, East Lansing, Michigan, 10-13 October 1974. Edited by Philipp Gerhardt, Ralph N. Costilow and Harold L. Sadoff. American Society of Microbiology, Washington 1975. $US15.00.

This volume of 619 pages contains 81 papers presented during four days by contributors from nine countries, with the United States, England, France and Japan predominating. It commemorates the upcoming centenary of the pioneer work on sporology by Ferdinand Cohn and Robert Koch, since the Seventh Conference is scheduled for 1977 or 1978 at the University of Wisconsin. It is noteworthy that there are no papers from Australia or New Zealand.

The binding of the volume, the paper, printing, photographic plates and diagrams are of the highest standard. There is an author index as well as a subject index. It is a pleasure to record that not a single typographical error was noted.

The book is divided into five sections, three major ones on Comparative Sporology, Sporulation and Germination and two shorter ones on Dormancy and Resistance, and General. Each paper is headed with an explicit summary.

Many of the photographs are electron micrographs and two plates of the spores of fungi showing freeze-etch replicas are of outstanding quality. A plate of black and white line diagrams of a bacterial spore contrasted with the extraordinary diversity, complexity and ornamentation of fungal spores is also impressive.

Under Comparative Sporology there are subsections on the groups of organisms — Actinomycetes, Myxobacteria, Azotobacter, Slime Molds, Blue-green Algae, Fungi and Yeasts with the two latter groups comprising 10 of the 19 papers.

The largest Section, that on Sporulation, consists of 37 papers devoted to the *Bacillus* group. Similarly the Section on Germination has 13 papers concerning the *Bacillus* group and one on *Clostridium botulinum*. The two shorter, final sections are equally dominated by the *Bacillus* group.

Because of this preponderance (and the natural interest of the reviewer), comment on individual papers not concerned with this one group would appear to be justified. The following are therefore selected for mention:

1. Actinomycete Spores: T. Cross and R. W. Attwell, England.

This is a lengthy review article with 133 references in the bibliography. It provides an invaluable reference for the meticulous description of spores of the bacteria included in the order *Actinomycetales*. The authors consider that work on the sporulation of these organisms has been overshadowed for too long by the amount of research devoted to the spores of *Bacillus* and *Clostridium*.

2. Spores of the Cellular Slime Mold Dictyostelium discoideum: David Cotter, U.S.A.

This paper is one of two dealing with a

somewhat neglected group of organisms. Four plates of finely detailed electron micrographs show the formation of spores and the emergence of the myxamoebae.

3. Diverse Spores of Fungi: D. J. Weber and W. M. Hess, U.S.A.

This is the paper with the remarkable plates referred to earlier and like (1) provides a review article on its topic. There are detailed descriptions of spore structure, contents and appendages followed by investigations on the metabolism of the spore.

4. Ascospores of Yeasts: R. R. Fowell. England.

This is a similarly constructed review article on the endospores of sporogenous yeasts — which are not often of medical significance. The author compares the bacterial spore and its simple structure with the yeast ascospores which vary in shape and surface sculpturing. The physiology of sporulation and the cytological and biochemical changes involved are described.

5. Production, Ultrastructure, and Germination of Candida albicans. Chlamydospores: Antonio Cassone, Nicola Simonetti and Vittorio Strippoli, Italy.

The authors report on the production of hyphal and chlamydospore development in *Candida albicans* using media incorporating N-acetyl-D-glucosamine. There are also interesting details of the changes which take place in chlamydospores on germinating.

6. Chromosomal Maps of Bacillus subtilis: Frank E. Young and Gary A. Wilson, U.S.A.

As a glimpse into the degree of complexity of genetic research on this species this paper is impressively daunting. The map requires a double page spread and listing of the designations of the genetic markers requires eight pages of tables.

While this book commands admiration throughout and many of the papers provide stimulating reading for an interested technologist, it is not likely to be of practical value for routine medical laboratories in New Zealand. The majority of contributors are from university research teams. Hence it is for libraries of institutes concerned with advanced teaching or research in microbiology that the volume should be recommended as an invaluable report of an important international conference.

F. M. Rush-Munro.

Acid-base and Electrolyte Balance, by Gösta Rooth. English Edition. 1975. Wolfe Medical Publications Ltd., London. $NZ7.15. N. M. Peryer Ltd., Christchurch.

Although this is a first edition it is based on two earlier works on the same subject and many people will remember the earlier versions. They contained a great deal of useful information simply and clearly expressed and this small volume brings the information up to date.

The author states in the introduction that his intention is to teach principles rather than to discuss clinical variations: however the various relationships are illustrated by referring to clinical situations. He has once again explained acid-base balance and disturbance in a direct and simple fashion.

The book begins by defining the terminology used and in order to make the reader familiar with SI Units both these and the older nomenclature are given in many instances. Acid-base balance is defined as the state achieved when H^+ concentration is kept within physiologically acceptable margins. This is essentially achieved by the buffering action of bicarbonate and protein. This is related to water and electrolyte balance.

Current methods for determining acid-base are discussed. The author likes to use the familiar "Gamblegram" to illustrate the anionic and cationic balance in various situations and recommends it as an aid to appreciating the interaction of water, electrolyte and acid-base concentrations. Siggard Anderson curves and alignment nomograms are also favoured for calculating acid-base parameters.

The various compensatory mechanisms involved in acidosis and alkalosis are discussed with clinical illustrations. Hyperosmolar nonketotic diabetic states and lactic acidosis receive mention in the chapter on diabetic acidosis. Renal insufficiency, salicylate intoxication and the effect of diuretics are also discussed. There are a few spelling mistakes and infelicities of grammar and style no doubt due to translation, but these detract very little from this introductory booklet which should be well received in the laboratory.

R. D. Allan.

Pathophysiology of Blood, by Alan J. Erslev and Thomas G. Gabuzda. Published by W. B. Saunders Company and obtainable

from N. M. Peryer Limited, Christchurch. 187 pages. Illustrated. Price $NZ9.40.

In order to discuss the role of blood it is necessary to look at the other elements and systems on which they depend. Red cells need the co-operation of the cardic output, pulmonary capacity, vascular reactivity and renal function to bring oxygen to the tissues: granulocytes need the support of plasma factors to carry out phagocytosis; lymphocytes produce and react with gammaglobulins; and platelets cannot be considered separate from the coagulation factors. It is with this in mind that the authors have attempted to correlate structure, function and kinetics of the blood cells with those of the other organs systems.

The book opens with short chapters on the bone marrow and spleen and then presents chapters on erythrocytes, phagocytes, immunocytes. thrombocytes, plasma coagulation factors and concludes with a chapter titled "Individual Identity of Blood Cells". Each chapter is introduced with a short section on structure, function and kinetics before discussing pathophysiology in more detail. Each section is clearly sub-headed and the book is written in a relaxed style which is often punctuated with some humorous terminology.

It is a book which bridges a gap between basic training text books and the many specialised books in haematology by stimulating the reader to pursue many of the listed references.

The book is well illustrated with graphs. tables, photomicrographs but is only protected by a light cover.

The authors have directed the book to medical students and haematology but it could also find a valued place in the hands of tutors teaching haematology to technologists and should prove valuable to trainees preparing for Part II and Part III examinations.

B. T. Edwards.

Book Reviews

Essentials of Medical Virology. Robert Wm. Pumper, B.A., M.Sc., Ph.D., and Herbert M. Yamashiroya, B.A., M.Sc., Ph.D. Publisher W. B. Saunders Company, Philadelphia, U.S.A., 1975. 278 pages. Price $7.65. N. M. Peryer, Christchurch.

"Virus studies please" is still a common request on forms accompanying specimens sent to diagnostic virus laboratories indicating that many physicians continue to have less than a vague knowledge of medical virology. Therefore any new text which could improve this situation must be welcomed, particularly by the laboratory workers who must interpret the virology request forms. Additionally an improved knowledge of virology must ultimately benefit the patient from whom specimens for examination are taken. Doctors Pumper and Yamashiroya from Chicago, U.S.A., have written a concise summary of medical virology to help all workers in virology.

There are 33 chapters with an average of seven to eight pages each. Each virus or group of viruses is dealt with under seven separate headings, viz., properties of the virus, host response to infection, diagnosis, treatment, epidemiology, prevention and control. The text is not illustrated except for one small schematic drawing of a virion.

Where knowledge of the antigenic properties of a virus are essential to understanding the pathogenesis of infection, e.g., influenza, added information is given. Practising physicians will find the clinical classfication of viruses extremely useful, i.e., a possible answer to the question "what viruses are likely to be involving the nervous system in this patient?"

Where applicable, up-to-date methods of treating viral infections (without precise details) are included.

The type of cell line in which various viruses grow is useful information for laboratory personnel.

Brief discussions on hepatitis, Epstein-Barr virus, compromised host and gastrointestinal virus infections remind readers of recent advances in these subjects.

The book is soft covered and the print is photocopied from a typed manuscript on good quality paper. For many users this type of reproduction is monotonous and difficult to read. On the other hand, when the book is used as designed. i.e., as a quick reference in the physician's surgery or by the laboratory worker at the bench, the type of printing is probably acceptable. Considering 1975 book costs, this text at $7.65 is good value.

Obviously, for the description of the more unusual and rare viral syndromes and for precise details of laboratory techniques more detailed works must be consulted, but "Essen-

tials of Medical Virology" can be fully recommended as a quick, up-to-date summary for all workers and students in medical virology.

E. R. Smith.

Comprehensive Review for Medical Technologists, 1975. Edited by A. M. Semrad. Published by C. V. Moseby Company. 206 pages. $NZ11.45. From N. M. Peryer Ltd., Christchurch.

The preface of this Review for medical technologists in the United States says that the book was written to help the reader review his academic background and that medical laboratory technologist education extends beyond the performance of laboratory procedures to their correlation and interpretation. It was compiled by a number of doctors and technologists and consists of a series of questions in the various sections with fairly full and detailed answers. The questions are often used as a basis for discussing the topic and are often clinically orientated and are selective rather than comprehensive. It is not a large book. Chemotherapy is rather neglected in microbiology and references are omitted.

The immunohaematology section has some deficiencies. The differentiation of A_x and A_m is incomplete. Of the other blood group systems no mention is made of the Ss system, outdated terminology is used for the P system and there are incorrect entries in the Lewis and Colton systems. The preparation of blood products such as platelet concentrate is scarcely covered.

The clinical chemistry is restricted to lipids, proteins, carbohydrates, hormones and enzymes and electrolytes. There is also a chapter on instrumentation and quality control.

The consensus of the reviewers was that this was an interesting book on an aspect of medical laboratory technology usually ignored; however, the answers to the somewhat random questions were not particularly profound and in general more applicable to the intermediate level of examination attainment.

D. S. Ford, B. W. Main, H. C. W. Shott and A. G. Wilson.

Handbook on the Laboratory Mouse. Charles G. Crispens Jr., Ph.D., 1975. 267 pages. $US9.75. Charles C. Thomas, publisher, Springfield, Illinois, U.S.A.

This handbook is a well-organised series of tables covering inbred strains of mice, their genetic attributes, genetic diseases, and uses. It will be most useful to those with some knowledge of mice and genetics, due to a lack of explanatory information. Sections include genetics, reproduction and development, blood, excretion, respiration, infectious diseases, neoplastic diseases, non-infectious diseases and a section on behaviour, fitness, immunology, etc.

The users will find the sections on blood and infection the least useful due to the variations in methods of haematology and the superficial coverage given to infectious diseases. However, these are not serious defects since the handbook is well referenced, making it a useful starting point for a number of areas.

Both general and specific references appear to have been chosen with great care to give the user a broad range of supporting and explanatory information.

Errors are few in number and appear to be of typographical origin.

This handbook should find extensive usage in any laboratory devoted to mouse research or genetics. Gerald L. Coleman.

Colour Atlas of Histology. M. B L. Craigmyle. Published by Wolfe Medical Publications Ltd., London. Price $NZ24.20. 320 pages, mostly illustrations. From N. M. Peryer Ltd., Christchurch.

This book, which is one of a comprehensive series on biology and human pathology, consists of 318 pages of photo-micrographs of body tissues with accompanying notes and measures 20cm × 14cm × 2.75cm.

The few introductory pages on staining methods are perhaps somewhat brief but the author appropriately points out that complete coverage of this topic is beyond the scope of this book. The remainder of the text taken up by various sections, each covering a body tissue grouping with concise notes at the beginning of each section which adequately cover the basic features of the tissue under discussion, and the complimentary photomicrographs follow — these having an accurate description. Occasional minor discrepancies occur throughout the text, for example, the statement that lymph nodes and spleen do not possess epithelial cells would be disputed by most histologists. However, this minor

feature is greatly overshadowed by the quality of histological staining and photo-micrographic reproduction with which it is an impossible task to find fault. Particularly noteworthy are the sections on epithelium, the female reproductive tract, the auditory apparatus and bone development. A total of 547 photo-micrographs, all in full colour, are found in this book. The author states "that this book is aimed primarily at undergraduate students of medicine and dentistry". Perhaps more importantly it will fill a void on the book shelves of those studying biological sciences, including medical technology. The author and Mr L. Jones, Chief Technologist, Department of Anatomy, University College, Cardiff, are to be congratulated with the publishers on this production, which is not only a scientific text but in itself a work of art.

Bert White.

Abstracts

Contributors: D. G. Bolitho, Lexie Friend, L. M. Milligan and Janice Parker.

Clinical Biochemistry

Single Channel Autoanalyser Adapted for Multiple Tests without Manifold Changes. Z. K. Shihabi (1975), *Clin. Chem.* **21**, 1567.

Multiple tests are performed on a prefixed set of tubing by altering initial reagent concentrations. Thus calcium, uric acid, glucose, phosphorus, chloride, BUN and CO_2 estimations can be performed on a single channel analyser without the necessity of changing manifolds. A list of reagents and conditions is included. —J. P.

Device for More Economical Standby Operation of Continuous Flow Analysers. Stephen C. Wardlaw (1975), *Clin. Chem.* **21**, 1667.

A device which decreases effective pump rate of a continuous flow analyser to less than 1/15 normal by cycling the pump periodically. Its use precludes leaving the instrument on full-time for emergency analyses and at the same time removes the necessity for repriming. The author has found a considerable resultant saving in reagents. —J. P.

Use of the Reference Method for Determination of Serum Calcium in a Quality-Assurance Survey. Pickup *et al.* (1975), *Clin. Chem.* **21**, 1416.

The authors examine the relationship between survey participants' results and the corresponding value obtained by the Reference Method. This identified methods with particularly discrepant results, and the reference method was used to assess the accuracy of field methods. The authors found methods using EDTA to be completely unacceptable. —J. P.

Studies on the Stability of Pancreatic Enzymes in Duodenal Fluid to Storage Temperature and pH. E. F. Legg and A. M. Spencer (1975), *Clin. Chim. Acta* **65**, 175.

The effects of storage pH and temperature on pancreatic amylase, lipase and trypsin in duodenal fluid were studied. All the enzymes were most stable when stored at —20°C, while the optimum storage pH was found to depend on the particular enzyme under investigation. —Authors' Summary.

The Unsuitability of Creatinine Excretion as a Basis for Assessing the Excretion of other Metabolites in Infants and Children. D. A. Applegarth and P. M. Ross (1975), *Clin. Chim. Acta* **64**, 83.

The authors found the normal value of excretion of a metabolite expressed relative to creatinine to be falsely age dependent and misleading during the first 10 years of life. —J. P.

Chemical Inhibition Method for Alkaline Phosphate Isoenzymes in Human Serum. O'Carrol, D., Staltland, B. E., Steele, B. W. and Burke, M. D. (1975), *Amer. J. clin. Pathol.* **63**, 564.

The authors adapted a chemical inhibition procedure using L-phenylalanine and urea as specific inhibitors to quantitate the activities of bone, liver, and intestinal alkaline phosphatase (ALP) isoenzymes in human serum. The procedure proved to be an acceptable way to clarify the source of elevated ALP in serum. —L. R. F.

CAP Survey to Assess the Extent of Stray Light Problems in Precision Spectrophotometry. Beeler, M. F. and Lancaster, R. G. (1975), *Amer. J. clin. Pathol.* **63**, 959.

From the article. "When the absorbance at 240nm of a solution of sodium iodide was measured by 159 laboratories using narrow-band-pass spectrophotometers, 15 percent reported results that suggested a problem caused by stray light."

The survey concludes with advice on how to remedy this problem. —L. R. F.

Progress and Analytic Goals in Clinical Chemistry. Gilbert, Roger K. (1975), *Amer. J. clin. Pathol.* **63**, 960.

A review of the data accumulated by the Survey programme of the College of American Pathologists for the analysis of 12 serum constituents for the five-year period 1969 - 1973.

A worthwhile review for those laboratories who have taken part in this survey. —L. R. F.

An International Laboratory Survey. Skendzel, L. P., and Copeland, B. E. (1975), *Amer. J. clin. Pathol.* 63, 1007.

Laboratories throughout the world were sent eight lypholised aliquots of human sera, and eight liquid samples. The sera were analysed by the participants for glucose, calcium, cholesterol, urea nitrogen, sodium and potassium. The liquid samples had haemoglobin and haematocrit assays performed by the participants. At the same time, over 4,000 laboratories in the United States analysed the same samples.

The results suggest that laboratory testing in the international group follows the pattern seen in the United States. "Despite its limitations, this study represents an initial effort to define differences in laboratory testing, on an international level at a time when such data are scarce." —L. R. F.

The Baton Dialyser. Saravis, Calvin A. (1975). *Amer. J. clin. Pathol.* **64**, 236.

An inexpensive efficient dialysis device for handling macromolecular solutions, e.g., radioactive materials, sterile solutions or infectious materials, is described. Solutions such as these which require special handling precautions for dialysis, may be safely and easily handled using this Baton dialyser. —L. R. F.

Monoclonal Gammopathies and Neoplasia. Makler, M. T., Uyeda, C., and Parkash, A. (1975), *Amer. J. clin. Pathol.* **64**, 706.

A report of a study to determine the incidence of monoclonal gammopathies (MG) in a hospital population. The sera were firstly examined by cellulose acetate electrophoresis. Of the 11,275 patients ranging in age from 20 to 84 years, 95 sera contained an MG, an incidence of 0.84 percent.

The report concludes with a recommendation that when a monoclonal protein is present, before classifying it as a benign MG, a thorough clinical and laboratory examination is warranted to rule out the possibility of an occult neoplasm. —L. R. F.

Haematology and Immunohaematology

On the Probability of Finding an HL-A and ABO Compatible Cadavar Organ for Transplantation. Pliskin, J. S. (1975), *Transplantation* **20**, 163.

This article looks at the probabilities of finding an HL-A and ABO compatible cadavar organ for transplantation. The results presented give a practical view of how long it would take to find a specific and compatible organ that is suitable for transpiant. —L. M. M.

A Simple Method for the Prediction of ABO Incompatibility using Sephadex A-50. Graham, H., Morrison, M. and MacAndrew, R. (1975), *Vox Sang.* **29**, 317.

A rapid and simple method of demonstrating a high titre of IgG Anti-A or Anti-B is described. DEAE Sephadex A-50 is used to separate IgG anti-A/B from IgM anti-A/B using a simple spin technique. The IgG fraction remaining is then titred. —L. M. M.

A Protocol for Cryo-precipitate Production. Burka, E. R., Harker, L. A., Kasper, C. K., Kevy, S. V., Ness, P. M. (1974), *Transfusion* **15**, 307.

A report and discussion concerning results achieved during a three-year research programme. —L. M. M.

Determinants of Factor VIII Recovery in Cryoprecipitate. Kasper, C. K., Myhre, B. A., McDonald, J. D., Nakasako, Y., Feinstein, D. I. (1975), *Transfusion* **15**, 312.

Many aspects of the production of cryoprecipitate were studied to determine which methods resulted in the greatest recovery of Factor VIII. A number of recommendations resulted which should improve the yield of Factor VIII. —L. M. M.

Chemical and Haematological Changes in Stored CPD Blood. Bailey, D. N., Rove, J. R. (1974), *Transfusion* **15**, 244.

Results of analysis carried out are presented and discussed. A number of units were bled into CPD and stored at 4°C. The following levels were measured at 1, 2, 7, 14, 21 and 28 days of storage: Plasma dextrose, sodium, potassium, chloride, bicarbonate, GOT, LDH, haemoglobin, WBC, MCV, PCV, MCHC, whole blood pH and ammonia. An interesting fact to emerge was that routine transportation, processing and handling of blood may lead to increased biochemical alteration. L. M. M.

Microbiology

Variants of *Escherichia coli* giving the appearance of mixed growths in urine. Nichols, G. L. (1975), *J. clin. Path.* **28**, 728.

A description of three strains of *E. coli* which gave the appearance of mixed growth on culture and were found on investigation to be variants of single strains, the mixed appearances being caused by mutation or phage action. The author points out the importance of this observation in examining mid-stream urine specimens as such specimens may be wrongly rejected as being mixed growths due to faulty collection. —D. G. B.

Incidence of *Listeria monocytogenes* in nature. Weis, J., and Seeliger, H. B. R. (1975), *Applied Microbiol.* **30**, 29.

This paper suggests that *Listeria monocytogenes* is a saprophyte which probably lives in a plant and soil environment. Such a habitat would make it easy for humans to contract the organism by several routes and from a variety of sources. The evidence on which this hypothesis is based is that of a research project during which 194 strains were isolated. 154 of these from soil and plant samples. —D. G. B.

Comparison of Antibiotic Disks from Different Sources. Brown, B. F. J. and Kothari, D. (1975), *J. clin. Path.* **28**, 779.

The paper stresses the marked differences which are obtainable in zone sizes with diffusion and sensitivity tests. It was notable that the disks from English manufacturers (Oxoid and Mast) gave greater zones of inhibition than equivalent Swedish or American disks. This paper shows once again the difficulties which can attend the Kirby-Bauer method of antibiotic testing. It would seem to point to the greater suitability of agar dilution or the Stokes method of testing for antimicrobial sensitivity for the smaller laboratory. —D. G. B.

Septicaemia due to *Corynebacterium haemolyticum* Jobanputra, R. S. and Swain, C. P. (1975), *J. clin. Path.* **28**, 784.

A rare human case of septicaemia due to *Corynebacterium haemolyticum* is described. Serological and bacteriological evidence for infection were present. Once again this report illustrates the importance of not rejecting "Diptheroids" from unusual sources as mere contaminants. —D. G. B.

A Clinical Isolate of *Salmonella typhi* requiring Anaerobic Conditions for Primary Isolation. Huber, T. W., Macias, E. G., Holmes, P., Bredthauer, H. D. (1975). *Amer. J. clin. Path.* **63**, 117.

This paper describes the isolation and characteristics of an *S. typhi* strain which was isolated from the blood culture of a 10-year-old boy and which initially grew only under strict anaerobic conditions. Even after several serial subcultures the organism still required CO_2 supplement for growth.

—D. G. B.

Interpretation of the Tube Coagulase Test for the Identification of *Staphylococcus aureus*. Sperber, W. H. and Tatini, S. R. (1975). *Applied Microbiol.* **29,** 502.

The authors offer a convincing argument to show that only if the coagulation of plasma after overnight incubation is sufficiently firm for the tube to be inverted without disruption of the clot can the coagulase test be interpreted as positive. Evidence is offered that lesser degrees of clotting is not necessarily indicative that the organism is *Staphylococcus aureus*. Variable results obtained with different types of coagulase plasma are discussed. Those laboratories using the coagulase test should be aware of the findings of this paper.

—D. G. B.

The Effects of Bicarbonate on Growth of *Neisseria gonorrhoeae* Replacement of Gaseous CO_2 Atmosphere. Cowley, R. S. and Baugh, C. L. (1975). *Applied Microbiol.* **29,** 469.

This paper describes experiments which demonstrate that the requirement for an atmosphere with a raised CO_2 content can be eliminated if sodium bicarbonate is incorporated in culture media. This may be of considerable use to those laboratories receiving "transgrow" plates from clinics at a distance.

—D. G .B.

Identification of the Yeast-like Fungi from Systemic Infections. Boyd, E. (1975). *Med. Lab. Technol.* **32,** 115.

An identification scheme for yeast-like fungi is described. The scheme uses direct microscopy, the germ tube test, a test for chlamydospore and mycelium production with carbon five as simulation tests using four carbohydrate fermentation tests plus a urease test. It is claimed that these 13 tests enable most common yeast-like fungi to be readily differentiated.

—D. G. B.

Coagulase negative Staphylococci and Micrococci in Urinary Tract Infections. Meers, P. D., Whyte, W. and Sandys, G. (1975). *J. clin. Path.* **28,** 270.

The authors examined 100 catalase positive, coagulase negative Gram positive cocci isolated in significant numbers from urinary tract infections. Their findings confirmed those of Kerr (J. clin. Path. 26: 918-920 1973) and showed that strains of Micrococcus group 3 were urinary tract pathogens, particularly in young women. The authors give a full summary of techniques available for differentiating these organisms and suggest that a rapid technique for the differentiation of Micrococcus type 3 from other coagulase negative staphylococci and micrococci is the use of a 5 microgram disk of novobiocin. Micrococcus type 3 species being resistant to this antibiotic, while other micrococci and *Staphylococcus epidermidis* strains are sensitive to this antibiotic.

—D. G. B.

Re-evaluation of Bacteriocinogeny in Neisseria gonorrhoeae. Skerman, J., Knapp, S. F. and Homes, K. K. (1975). *J. clin. Path.* **28,** 274.

This paper confirms the findings of previous investigators that gonocin typing is not a useful test.

—D. G. B.

Correspondence continued

Sir,—It would seem that, in delivering the T. H. Pullar Memorial Address at last year's Conference, I may have allowed myself to get a little carried away in expressing the views I deemed appropriate at the time. That, of course, is always the trouble with rhetorical exaggeration on these occasions. Though the spoken word falls but fleetingly on listening ears before being dispersed by the winds, its rendering in print bestows on it a kind of permanent authority that, if not entirely accurate, may invite an indignant challenge. That such a challenge should have resulted from your publication of my remarks in the November 1975 issue of the *Journal* is no more than deserved, I suppose, and it is no more than right that I should endeavour to set the record straight.

The observation to which exception has been taken, and concerning which I now appear before you attired in penitential sackcloth and ashes, is that concerning the reclassification of holders of the Fellowship Diploma as "Technical Officers". I confess that I may well have allowed myself to be misled by the gloomy expectations of individuals when the current New South Wales Awards were written, for it seems that in no authenticated case has the event mentioned actually taken place. The Awards concerned specify separate definitions for the occupational classes of "Scientific Officer", "Medical Technologist" and "Technical Officer", and to suggest that these might be interchangeable is plainly in error. Not that this retraction negates the main objective of my remarks, which was to caution that the restructuring of a training course, and any consequent upgrading of the resultant qualification, is inevitably attended by concern on the part of existing practitioners about the effect of the measure on their own status and future. That provision should also be made to safeguard their interests is only just, whether this consists of a bridging course, a grandfather clause or a combination of the two.

John Case.

Obituary

A. Fischman

On Tuesday, February 10, 1976, the sudden and untimely death occurred of Andor Fischman. Up until his retirement on June 29, 1975, Andor was Charge Technologist, Department of Immunology, Auckland Hospital.

Andor was born in Budapest, Hungary, 1910, and completed his education there finally graduating Doctor of Laws. Following this he worked in a hospital at both radiography and laboratory technology to prepare himself for a future career in New Zealand, a decision taken in response to the conditions prevailing in central Europe at the time. He arrived in New Zealand in 1938 and after working for a time on a chicken farm, he joined his brother, Dr E. J. Fischman, an Auckland physician. Together they embarked on a series of investigations into blood protein fractions, tests of thyroid function, etc. During the next few years, they collaborated on a number of projects, culminating in a series of articles in overseas and local scientific journals.

In June 1953, Andor commenced technology training in the Auckland Hospital laboratory and qualified in February 1957. He subsequently took up the position of Charge Technician of the Serology section, as it was then called, a position which he held until his retirement.

During his years of involvement in this field, Andor made major contributions to the technology of syphilis serology, of diagnostic rheumatoid arthritis serology, and hydatid disease. In the latter two, he was responsible for the development of highly successful laboratory methods for which he received deserved world recognition. His contribution in this field was immense, and his influence on the standard and scope of diagnostic serology in this country incalculable. The list of his published articles is long and includes items in many of the more important scientific journals.

While he never held office in the New Zealand Institute of Medical Laboratory Technology at either branch or national level, Andor took a vital interest in Institute affairs. His legal training enabled him to analyse and criticise procedural affairs, often to their betterment. He was one of the strongest advocates for the establishment of branches and was always greatly concerned about the status of the profession. His presentation of papers and talks at Institute meetings throughout his working life were all of a very high standard, and always displayed to its best advantage his quiet sense of humour.

In private life Andor Fischman was a deeply religious man. He took an active part in the work of the Auckland Jewish community. Among other things he gave lectures, took part in seminars and wrote articles for New Zealand Jewish magazines. His main concern was Jewish/Christian dialogue. He made a special study over many years of Professor Martin Buber (1878-1965) the great philosopher who had left Germany in 1938 to live in Israel where he became a lecturer at the Hebrew University of Jerusalem.

On one of his visits to Israel, Andor had the honour of meeting Martin Buber, and after his retirement he worked intensively on a study of the Professor's philosophy. He had hoped to publish the results of his work in the near future, but it was never completed. His papers are being sent to the Hebrew University library.

Andor Fischman epitomised the thorough scientific approach to laboratory technology and a quiet, sincere approach to life in general to which we would all aspire but few achieve.

"Zichrono livracha"
("May his memory be a blessing".)

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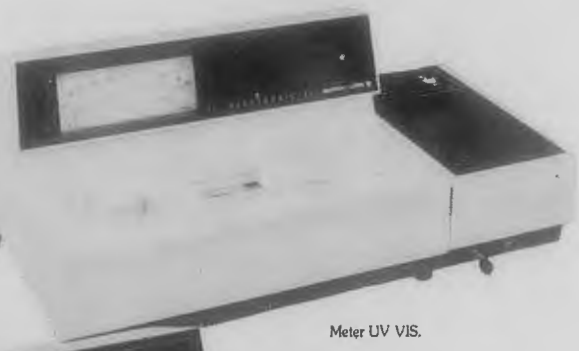

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Illustrations are costly and should be used sparingly. Graphs, line drawings and photographs are all referred to as 'Figures' and should be numbered in the order of their appearance in the text using arabic numerals. Drawings (in indian ink on stout white paper) and photographs, should be about twice the size of the actual reproduction. The position of figures in relation to the text should be noted in the typescript. Legends typed on separate sheets are numbered to correspond with the illustrations. Tables should be typed separately and numbered in roman numerals.

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