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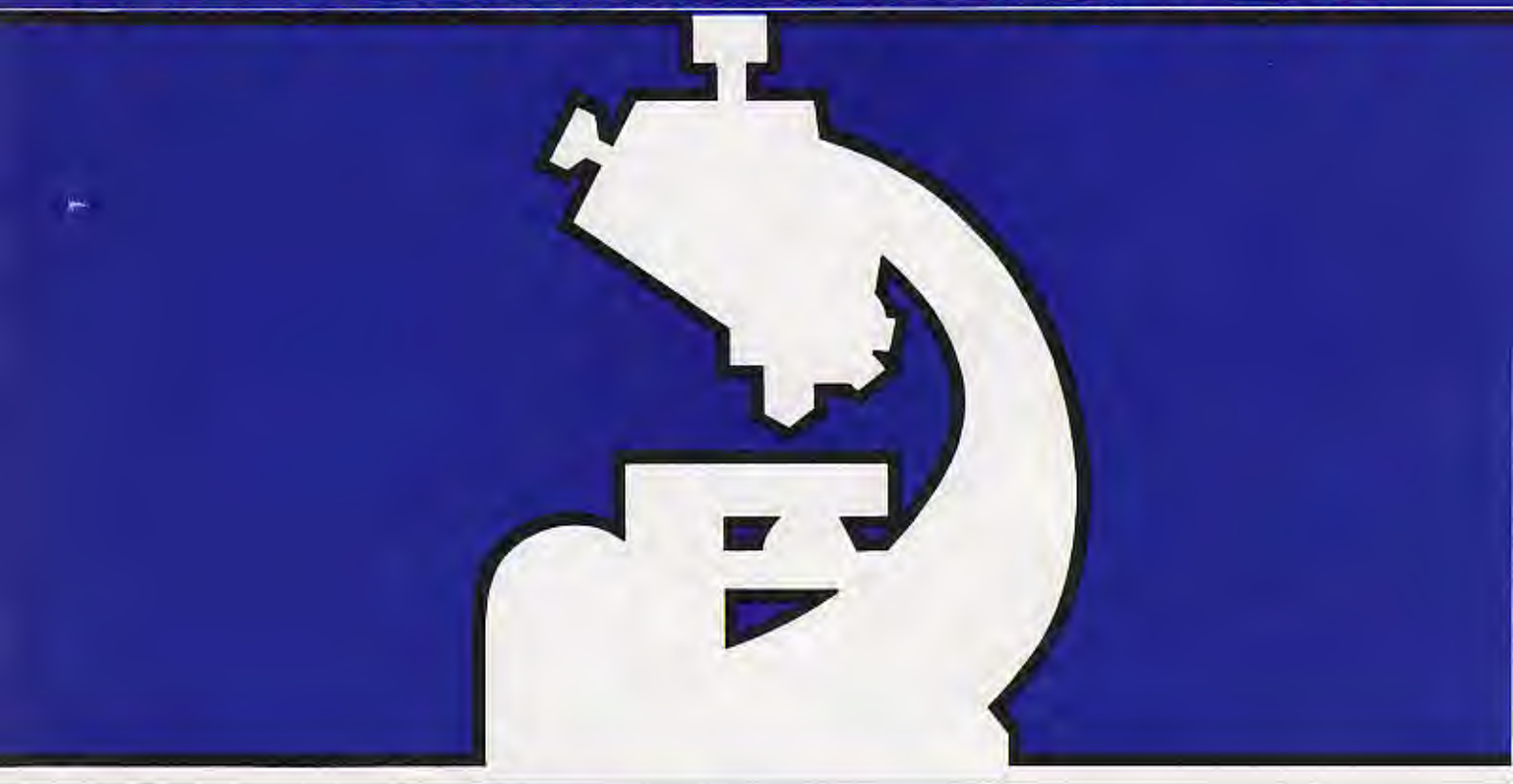

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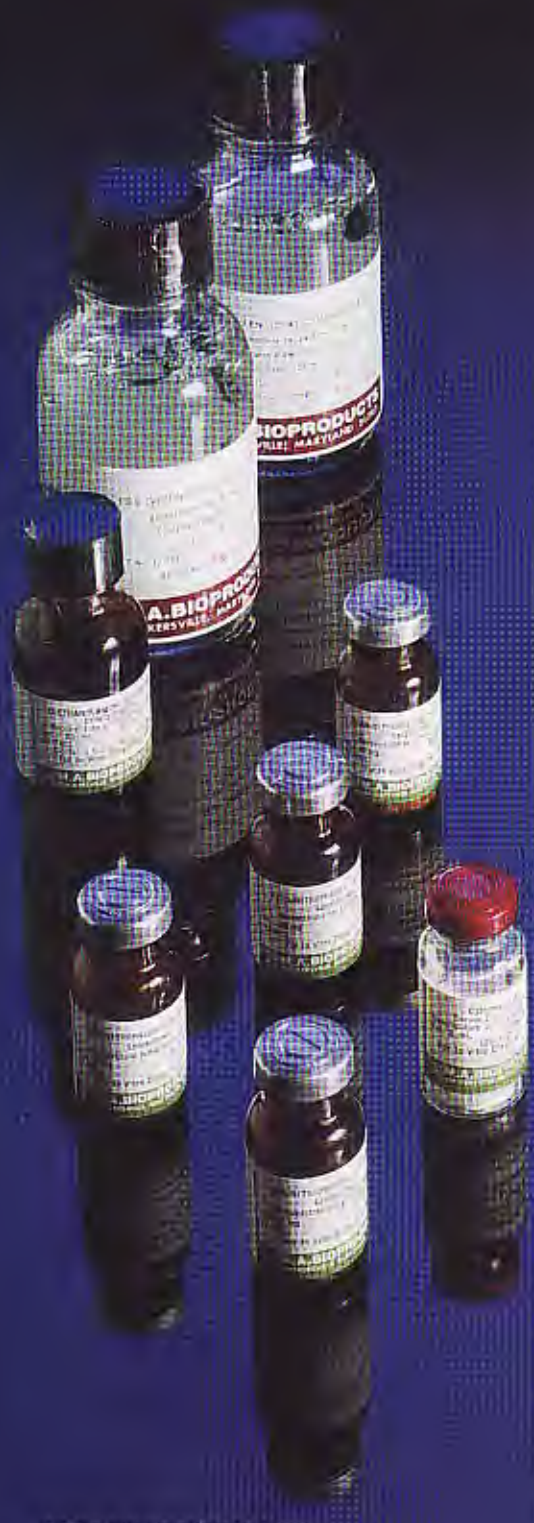

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The Lupus Anticoagulant — A Review

Allan S. Johns
Haematology Dept, Greenlane Hospital

Abstract

A review of the current literature on the lupus anticoagulant with reference to the aetiology, mechanism of action, its association with bleeding, thrombosis, recurrent intrauterine death, and high foetal wastage. A discussion of laboratory tests available for screening these patients together with recommendations from the author's laboratory of suitable tests to detect and categorize the inhibitor.

Introduction

The Lupus Anticoagulant (LA), first described by Conley and Hartman[1] in 1952, in a patient with Systemic Lupus Erythematosus (SLE) is a spontaneously acquired inhibitor of coagulation that interferes with the activation of the Prothrombin activator complex (factor Xa, factor V, calcium and phospholipid)[2] by inhibiting the phospholipid portion of the complex. The inhibitory activity of this protein is of a heterogeneous nature and inhibitory activity has been reported directed against fibrin polymerisation [3], activated factor X[4], factors IX and XI[5], factors XI and XII[6], factor II[7], and against one of the contact activation factors Prekallikrein (Fletcher Factor)[8]. Both quantitative and qualitative platelet defects have been reported in patients with the LA[9] and these together with hypoprothombinaemia are cited as causes for bleeding in patients with SLE.

The LA can not only be demonstrated in 5-10% of patients with SLE[10], but also in many patients undergoing psychiatric treatment with chlorpromazine [11], a variety of autoimmune and connective tissue disease, systemic vasculitis, polyarteritis nodosa, primary sicca syndrome, discoid lupus, Behcet's syndrome and systemic sclerosis. It has been demonstrated in women who present with consecutive unexplained intrauterine deaths[12], thrombotic episodes and delivery of growth-retarded infants, in other disease states (Neurologic, Cardiovascular, Oncologic and Urologic), and finally in many patients without an identifiable disorder.

Many patients with the LA have a positive Wassermann reaction, often termed a biological false positive test for syphilis[13], and circulating auto antibodies directed against lipid antigens such as cardiolipin can be demonstrated. Harris et al[14] suggest these immunological relationships are based on the presence of antibodies cross-reacting against cardiolipin (the phospholipid antigen detected in standard tests for syphilis, phospholipids in platelet membranes, and a phospholipid in the prothrombin activator complex of the coagulation cascade against which the anticoagulant acts. Finally, many cases of paradoxical thrombosis have been reported in patients with the LA often without any underlying disease[15].

Aetiology and Action of the Lupus Anticoagulant

The precise aetiology of the LA is not known but it has been shown that antibodies may cross-react with complex lipid antigens and monoclonal anti-DNA antibodies, raised from lupus mice, and may bind a variety of polynecleotides and phospholipids[16,17,18]. LA activity has been demonstrated by these antibodies. In such antibodies the antigenic determinants were shown to be the phosphodiester-linked phosphate group present in some phospholipids, including cardiolipin, as well as in the sugar phosphate backbone of polynucleotides such as DNA[16,17,18]. Certain restricted groups of lymphocytes may produce antibodies with idiotypic diversity. Support for this hypothesis come from Schwartz[18] who has shown that immunisation of normal rabbits or mice with cardiolipin stimulates the production of both cardiolipin and anti-DNA antibodies. The LA may be either IgG, or IgM or a mixture of both[9].

The activation of Prothrombin complex occurs on the surface of the platelet membrane lipid bilayer. Structural requirements of phospholipid activity in blood coagulation is minimal and mainly depends on the net negative charge on the phospholipoprotein surface or within the laminar aggregates or bilayers. It has been postulated that a molecule of factor Va is embedded in the lipid surface and is in 1:1 stoichiometry with factor Xa. Molecules of Prothrombin with the Prothrombin fragment 1, Prothrombin fragment 2 and Prothrombin 2 domains are either in solution or bound to the lipid surface through calcium ion bridging mediated by the gamma-carboxyglutamate residues of the fragment 1 domain[19]. The LA is thought to interfere with the calcium ion bridging of the Prothrombin fragments to the factor Va on the lipid surface. Hence, by reducing the phospholipid component of the clotting mixture, the inhibitors effect can be potentiated disproportionately. Loeliger [20] was first to describe a cofactor in normal plasma that was necessary for maximal action of the LA. This cofactor, which potentiates the inhibitory action of the LA, is responsible for the augmentation effect of this inhibitor. Augmentation is seen in some patients with the LA in whom the addition of small amounts of normal plasma may lead to the paradoxical prolongation of the Activated Thromboplastin Time or Kaolin Clotting Time.

The Lupus Anticoagulant and Bleeding

The majority of patients with the LA do not bleed abnormally and some have undergone major surgery (Open-heart surgery) without excessive intra- or post-operative bleeding. In a number of reports on patients with the LA who have had clinically significant bleeding, the haemostatic failure could be attributed to some other abnormality such as thrombocytopenia, depressed Prothrombin level or severe uraemia.

The frequent finding of the LA and hypoprothrombinaemia led some to postulate that the LA acts as an antiprothrombin[21]. Bajaj et al[22] investigated the hypoprothrombinaemia associated with the LA and confirmed the findings of other investigators that it is now clearly established that the plasma does not contain material capable of neutralising the coagulant activity of Prothrombin added in vitro[7,9,10], and that plasma Prothrombin antigen is decreased to the same extend as Prothrombin activity[9,23,24]. They also showed that the plasma of their patients with the LA contained antibodies that bind Prothrombin without neutralising its in vitro coagulant activity. From these results they postulated that the hypoprothrombinaemia in these patients arises from the binding of Prothrombin to Prothrombin antibodies in vivo. The clearance of these immune complexes from the circulation depends on the quantity and size. They further suggest that the decreased Prothrombin level is due to rapid clearance of Prothrombin antigen-antibody complexes. These findings are of great importance in the laboratory investigation of these patients as a screening test for antibodies by the inhibition of a functional assay system would detect a low prothrombin level, but not the presence of inhibition by the Lupus Anticoagulant. A similar mechanism acting on the other coagulation factors has not been demonstrated as yet.

Thrombosis and the Lupus Anticoagulant

The association of the LA and thrombosis is well established and a recent study by Boey et al[25] who studied 31 patients with SLE and other connective tissue disorders whose plasma showed the presence of a LA showed thrombotic episodes including deep vein thrombosis, pulmonary embolism, cerebral thrombosis and axillary vein thrombosis in 18 of the 31 patients. Thrombosis in patients with the LA has been reported in association with

thrombocytopenia[26], directed against factors XI and XII[27] and against factor VIII and factor IX.

Two mechanisms have been postulated to explain the parodoxical thrombotic tendency in this group of patients. Firstly, abnormalities in the fibrinolytic system, including abnormally low content of fibrinolytic activators in the walls of the superficial veins, and defective mobilisation of endogenous fibrinolytic agents, judged from abnormally low fibrinolytic response to standard stimuli such as venous occlusion. Astedt et al[28] have shown that fibrinolytic activity of the vein walls is markedly decreased during pregnancy and thrombosis during pregnancy, in association with the LA is well established. Inhibitory activity directed against Prekallikrein has been demonstrated. Patients plasmas exposed to kaolin failed to develop the level of fibrinolytic activity achieved by similarly treated normal plasma. As Prekallikrein is now recognised to be the plasminogen proactivator required in the factor XII dependant pathway of plasminogen activation[29] it may be postulated that compromised fibrinolytic capacity may be a contributing factor in the development of thrombosis in the these patients. Secondly, it has been shown plasma from patients with the LA do not generate Prostacyclin from fresh rabbit aorta[30]. It may be that the LA, by interfering with phospholipids, may inhibit the release of arachidonic acid (the substrate for production of Prostacyclin) from the cell membrane leading to a lack of inhibition of platelet aggregation and hence to a favourable prothrombotic state. Patients with the LA and thromboembolic disorders often demonstrate spontaneous platelet aggregation and increased sensitivity to low concentrations of aggregating reagents (personal observation). This may well be related to the inhibition of Prostacyclin release in these patients.

Obstetric Complications and the Lupus Anticoagulant

Recurrent spontaneous abortions in association with the LA have been reported by several authors [31,32,33,34,35]. Intrauterine deaths are usual in pregnant women in whom the LA can be demonstrated and thrombotic episodes in the placenta likely lead to fetal death[36]. Mechanisms responsible for this have been shown to be reduction of prostacyclin release from vessel walls [32,34] and from the human pregnant myometrium[32]. It is of interest to note that decreased production of Prostacyclin activity by human fetal[36] and maternal vessels[37], and low values of Prostacyclin activity in amniotic fluid[38], have been observed in patients with severe pre-eclampsia. This implies a probable relationship between the inhibition of Prostacyclin production by the LA and recurrent intrauterine deaths. A recent report[33] describes a patient with severe toxaemia of pregnancy, lupus anticoagulant, major thrombotic episodes involving leg veins and a subcapsular haematoma of the liver.

Clinical and serological evidence of SLE in a pregnant woman and a history of thrombosis or unexplained intrauterine deaths should arouse suspicion, and the woman be screened for the presence of the LA.

Laboratory Investigations

The LA is characterised by a prolonged Activated Partial Thromboplastin Time (APTT) and by a normal or mildly prolonged Prothrombin Time (PT). As the inhibition is directed against phospholipid, increasing the concentration of phospholipid will reduce the degree of inhibition, and reducing the phospholipid will increase the degree of inhibition. The latter technique is now often used as the basis of screening tests for the LA.

The working party on acquired inhibitors of coagulation of the international committee for standardisation in Haemostasis[39] put forward the following criteria for the diagnosis of the Lupus Anticoagulant:

1. Prolongation of the APTT.
2. The APTT of a 1:1 mixture with normal plasma should be greater than 4 seconds longer than the control.
3. A decrease in at least two factors (VIII, IX, XI or XII) by one-stage assay, with normal values by two-stage assay.
4. A sensitivity to dilution, so that the apparent activity of the factor increases with dilution.

Our experience with a number of patients with the LA do not support these criteria and will be discussed below.

The Activated Partial Thromboplastin Time

We have found that although the APTT is prolonged in the majority of patients with the LA it is often within the normal range in others in whom other screening tests are abnormal. The degree of correction varies considerably from patient to patient and may depend on the specific characteristics of the Anticoagulant and its action in each patient together with any associated factor deficiency. The major variable in the APTT test which can give widely discrepant results is not, as previously reported, the type of activator, but appears to be related to the source and dilution of the phospholipid used in the test. The working party on acquired inhibitors[39] reported that most currently available APTT reagents are adequate to diagnose more than 80-90% of patients with the LA. A study comparing 11 APTT reagents in current routine use[40] found a wide discrepancy between reagents with detection rates as low as 50%. This is similar to our findings and to date only one reagent appears to detect virtually all patients with the LA and that is bioMerieux Cephalite reagent which contains rabbit brain phospholipid and celite activator. Of interest is the fact that in this study, other APTT reagents also using rabbit brain phospholipid rated poorly and it now appears that the source of phosphlipid and the nature of the contact phase activator may not dictate the sensitivity to the LA and it could possibly be related to the critical concentration of the platelet substitute. This observation is supported by the observation by Exner et al[41] that the APTT is more sensitive to the LA by omitting the platelet substitute.

The Kaolin Clotting Time (KCT)

Developed by Margolis[42] the KCT is a Partial Thromboplastin time with kaolin in which the added platelet phospholipid has been omitted. This test is highly sensitive to the LA and Exner et al[41] have modified the test by introducing varying dilutions of patient plasma in normal control plasma to give four characteristic curves when clotting times are plotted against percent of patient plasma. The type one curve is described as the typical LA curve in which the addition of small amounts of normal plasma to the test system initially have no correcting effect. Type two probably represents the classical LA together with a factor deficiency which is quickly corrected by the addition of normal plasma. Type three pattern shown that mixing normal plasma with patient plasma results in more prolonged clotting times. This probably represents the absence in the patients plasma of the lupus-cofactor. Type four curves are obtained in patients with SLE but in whom no inhibitor is detected and addition of normal plasma to the patient's plasma has no effect on clotting times. We have found the KCT to be an excellent test for the detection of LA and it has always yielded a positive result when one or more other screening tests were positive. This test could, until more sophisticated technology supersedes it, be regarded as the reference test for the detection of the LA. For the KCT to be sensitive and reliable the plasma specimen must be platelet-free and this is best obtained by a double centrifugation technique with extreme care in handling, as any damage to platelets within the sample can lead to phospholipid release and hence to shorter, often normal, clotting times in an otherwise abnormal sample.

Platelet Neutralisation Procedure (PNP)

Increasing the concentration of phospholipid in a test system can overcome the inhibitory effect of the LA. A platelet suspension is prepared from a blood bank platelet concentrate and frozen in aliquots. In addition of platelet suspension to a prolonged APTT will result in a shortened clotting time in patients with the LA, but not in prolonged times in patients with specific factor inhibitors and hence forms the basis of a reliable screening test for the LA. One drawback of the test is it can only be performed reliably in APTTs that are moderately or markedly prolonged. In patients with only mildly prolonged APTTs the PNP test is difficult to interpret. The PNP cannot differentiate factor

inhibitors, either the LA or specific factor inhibitors, from the presence of heparin in the sample. As some patients being tested for the presence of the LA may be undergoing heparin therapy for thrombotic episodes, it is essential to ascertain if prolonged results are the result of heparin. This can easily be ascertained by the use of the Thrombin Clotting Time or other tests sensitive to the presence of heparin but not to the LA. Conversely, the presence of a LA in a patient receiving heparin can lead to difficulties in monitoring the heparin level as the APTT may be markedly prolonged before heparinisation. In these patients a heparin assay (Chromogenic) is probably the most reliable method of controlling the heparin therapy.

The Prothrombin Time (PT)

Only a small number of patients with the LA have an abnormal PT. The normal PT obtained with undiluted thromboplastin emphasises the relative insensitivity of this test to the LA and probably reflects the relative excess of phospholipid provided in the tissue thromboplastin. This can be overcome by diluting the thromboplastin, hence rendering it more sensitive to the LA.

The Dilute Tissue Thromboplastin Assay (DTTA)

This assay developed by Boxer et al[44] uses thromboplastin diluted exponentially to 10^{-4} as the thromboplastin source for the PT. Results are expressed as a ratio of a normal plasma also tested with the diluted thromboplastin. The assay is considered positive for the LA if the PT is prolonged 1.5 times the control plasma at a dilution of 10^{-3} and 1.7 times at a dilution of 10^{-4}. The assay was found to be within normal limits in patients with haemophilia, Christmas disease, von Willebrand's disease, patients with acquired factor VIII inhibitors and patients on coumadin anticoagulation, but was not reliable in patients receiving therapeutic heparin doses. In our experience, we have found the test to be positive in patients with high titre antibody levels but unreliable with low levels of antibody and because of this we do not use it as one of our diagnostic criteria for the LA.

Schleider et al[45] have modified the DTTA and use Simplastin (General Diagnostics) at a dilution of 1:50 and 1:500. The working party on Acquired Inhibitors[39] states that this modification, termed the tissue thromboplastin inhibition test TTIT, is approximately equivalent to the APTT in detection rate of the LA. That is, it is able to diagnose 80-90% of patients with the LA. This is not our experience and we have found it to be no better than the DTTA with a low detection rate. The DTTA and TTIT cannot differentiate specific factor inhibitors from the LA. Although Boxer[44] states that the DTTA is normal in Factor VIII deficiencies or inhibitors, there is no published data to support this.

Factor Assays

The report from the working party on acquired inhibitors[39] states that there should be a decrease in at least two factors (VIII, IX, XI, XII) by one-stage assay, with normal values by two-stage assay and a sensitivity to dilution, so that the apparent activity of the factor increases with dilution. Our experience does not support these criteria. We have found, in many instances, markedly prolonged KCT's and APTT's in patients with the LA but in whom normal assays were consistently found. In patients who do show low intrinsic factor assays the majority of patients do show sensitivity to dilution. One point of interest we have found in several patients, and which has been confirmed independently in other hospitals, is the paradoxical reverse sensitivity to dilution in factor assays in some patients with the LA. With increasing dilution we can demonstrate decreasing factor levels, and this is not related to technique and may be due to the type of phospholipid (Folch), or its concentration, used in the assay system. Some circulating inhibitors such as the LA may compete with phospholipids in the assay system for critical binding sites and excess phospholipid at low factor dilution may effect the phospholipid-factor interaction.

Platelet Aggregation Studies

Most reports on platelet aggregation defects in SLE have shown abnormalities similar to the release defect and indistinguishable from the pattern obtained after aspirin ingestion[46]. Although they could not demonstrate a correlation between the presence of antiplatelet antibody and impaired platelet function, they could show a correlation between the presence of a serum antiplatelet function factor(s) and poor platelet function. It is tempting to assume that this serum factor which results in poor platelet function is the LA. A patient with a LA directed against factors XI and XII[8] also showed massive spontaneous platelet aggregation but no evidence of thrombosis. We have encountered patients with the LA and thrombotic episodes in whom platelet aggregation defects, including spontaneous aggregation and increased aggregation to low concentrations of aggregating reagents, can be demonstrated. These defects are presumably related to the inhibition of Prostacyclin production and this group may respond to low doses of aspirin.

Tests of Fibrinolysis

Little work has been done on testing the fibrinolytic system[28,29] mainly because of the unreliability of many tests presently available to study this system. But with the advent of new methodologies, (i.e. chromogenic substrates), further studies will likely follow.

One test of limited use is the Kaolin-induced fibrinolytic activity test in which the time for lysis of clots formed from kaolin activation of patient or control plasma are recorded. In one study[8], patients with the LA showed longer lysis times than normal patients and the authors speculate that the fibrinolytic defect may result from the inhibition of Prekallikrein by the LA with subsequent compromising of the fibrinolytic system.

Recommendations for Haemostasis Screening of Patients

As a screening procedure for the detection of the LA in our laboratory we use the following protocol.

On double centrifuged, platelet poor plasma the following tests are performed:

- Activated Partial Thromboplastin Time using bioMerieux Cephalite and our APTT reagent in current use with immediate and incubated corrections if prolonged.
- Prothrombin Time
- Kaolin Clotting Time

If one or more tests suggests the presence of the LA we follow-up with a full lupus investigation on a fresh specimen, which includes:

- Above tests.
- Platelet Neutralisation Procedure.
- Kaolin Clotting Time and curve.
- Factor assays using at least 5 dilutions.
- Platelet aggregation studies using standard and low concentrations and testing for spontaneous aggregation.
- Dilute Tissue Thromboplastin Assay.
- More specialised tests including specific immunoglobulin characterisation and tests of fibrinolytic potential are occasionally performed if indicated.

Summary

During the 32 years since the first report of the LA, a vast fund of knowledge has amassed. The nature of the inhibitor, its site of action and its effect on many laboratory tests have been characterised. New tests have been developed to elucidate the inhibitor and most importantly its association with different clinical conditions has been established leading the way for more effective treatment and efficacious drug usage. Although the clinical syndrome will, probably in the future, be detected by the use of recently developed anti-tissue antibody tests, the role of the Haemostasis laboratory in detecting and characterising the LA and its effect on the haemostatic system will undoubtedly expand as new methodoligies proliferate. With the increasing number of clinical correlations the detection and accurate characterisation of the LA becomes vital.

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The Prevalence of Toxoplasma Antibodies among children in Hamilton

Ray T.M. Cursons, PhD, Scientific Officer, Sherryn Cepulis, Graded Laboratory Officer.
Pathology Department, Waikato Hospital, Hamilton.

Abstract
A survey of 161 sera from hospitalized children between the ages of 0.5 - 15 years established that 41.6% possessed antibodies to *T.gondii.* 13% of the children possessed antibody titres of ⩾1 : 512 and 4.3% had accompanying IgM titres of ⩾1 : 16.

Introduction
Toxoplasma gondii is an uniquitious protozoan parasite of man and animals. Infection by this protozoan occurs primarily from ingestion, either directly or indirectly via contaminated soil, of the mature oocyst present in infective cat faeces, or from the consumption of undercooked meat containing Toxoplasma cysts. Although infection by *T.gondii* is usually subclinical in the normal host, it can be fatal or devastating in the immunologically compromised or congenitally infected host[1,2,3].

The prevalence of antibodies to *T.gondii* in man increases with increasing age. Approximately 50% of the population in the United States possess antibodies to *T.gondii* and the incidence in Britain has been shown to progressively increase from 8.3% for the 0-10 year age group to 49.96% for the >-60 year age group.[2,3] A recent New Zealand study has shown the incidence to vary from 32.41% for the under 20 year age group to 50.32% for the >50 year age group and the prevalence of antibodies of *T.gondii* in pregnant women attending the Waikato Hospitals' Antenatal Clinics was shown to vary from 58.5% for the 15-20 year age group to 68.5% for the 31-55 age group.[4,5] It was therefore decided to investigate the prevalence of antibodies to *T.gondii* in the hospitalized paediatric age group, 6 months - 15 years, to observe the prevalence of this parasite in the paediatric community.

Materials and Methods
Test samples: Antibodies to *T.gondii* were assessed in the sera of children hospitalized for a variety of reasons. There was no selection for children with symptoms of PUO, lymphadenopathy or mononucleosis syndrome. Antibody levels were assessed in duplicate via the indirect haemagglutination test (IHA) and the indirect fluorescent antibody test (IFA)[6]. Children whose age was <6 months were not surveyed because of the probability of detecting maternal antibody. A serum was regarded as positive if the titre was ⩾1 : 64 for the IHA test and/or ⩾1 : 16 for the IFA test. An antibody level of ⩾1 : 512 was regarded as suggestive of recent infection. Group specific IgM antibodies were assessed by IFA using sera fractionated by column chromotography to remove competing IgG antibodies, antinuclear antibodies and rheumatoid factor.[2,6] Because of the dilution factor associated with column chomatography, the lowest IgM titre tested was 1 : 10. Serological results from quality assurance programs sent out by the Australasian College of Pathologists and the National Health Institute during the study period suggested that all serological test results were acceptable.

Age Group (years)	Number in group	% of study population	Number positive	% positive for antibody
0.1 — 4	38	23.6	9	23.7
5 — 10	65	40.4	29	44.6
11 — 15	58	36.0	29	50.0

The Prevalence of antibody to T gondii in 161 children
Table 1

Age Group (years)	Nos of sera tested	% of sera giving reciprocal titres of <64	64	128	256	512	⩾1024
0.5 — 4	38	81.6	5.3	7.9	2.6	2.6	0
5 — 10	65	64.6	6.2	7.7	6.2	7.7	7.7
11 — 15	58	60.3	8.6	8.6	6.9	13.8	1.7

The distribution of IHA antibody according to age
Table 2

Results
Of 161 sera examined 41.6% were found to have either an IHA titre of ⩾1 : 64 or an IFA titre of ⩾1 : 16 to *T.gondii.* Table 1 shows the age distribution and occurence of antibody positive individuals. The prevalence of antibodies to *T.gondii* was found to approximately double between the ages 4 and 10, plateauing out to 50% for the 11-15 year group. Tables 2, 3 and 4 show the distribution of antibody titres of the different serological tests in relation to age. Of the 67 children with positive antibody, 21 have titres of ⩾1 : 512, suggestive of infection as distinct from exposure. Seven children were shown to have IgM titres of ⩾1 : 16.

Age Group (years)	Nos of sera tested	% of sera giving reciprocal titres of <16	16	64	128	256	512	⩾1024
0.5 — 4	38	76.3	10.5	5.3	0	7.9	0	0
5 — 10	65	55.4	13.8	9.2	1.5	6.2	12.3	1.5
11 — 15	58	50.0	12.1	8.6	8.6	8.6	5.2	6.9

The distribution of IFA antibody according to age
Table 3

Discussion
The results confirm the direct correlation between the prevalence of antibodies to *T.gondii* and increasing age as described in other serological surveys.[1-5] Relatively few hospitalized children under the age of 4 years possessed antibodies to *T.gondii* but thereafter the prevalence was observed to substantially increase with a doubling of the prevalence between 4 and 10 years. Altogether 41.6% of the children sampled had seroconverted to *T.gondii* with 13% possessing a titre of ⩾1 : 512, suggesting infection as distinct from exposure. Twenty of the 21 children possessing a titre of ⩾1 : 512 were in the 5 — 15 year groups, the age groups which also had the highest rate of seroconversions. Of these 21 children, 33.3% also possessed IgM titres of ⩾1 : 16. The variation in titres observed between the IHA and IFA results from the different antibodies measured by these tests. Whereas the

Age Group (years)	Nos of sera tested	No of sera giving reciprocal titres of <8	8	16	32	64	256
0.5 — 4	1	1	0	0	0	0	0
5 — 10	10	9	0	1	0	0	0
11 — 15	10	4	0	1	2	1	2

The distribution of IgM antibody according to age
Table 4

former technique detects antibodies against soluble cytoplasmic antigens which appear later in the course of the disease, the IFA test detects early antibodies directed against surface antigens. [2,6].

Whether the same seroconversion rate to *T.gondii* occurs in the normal paediatric population is largely unknown at this stage. Although every attempt was made not to prejudice the population sampled, the population nevertheless is biased. Consequently it is difficult to extrapolate these results with respect to the unhospitalized population. However, two previous serological surveys of the Hamilton population have recorded seroconversion rates to *T.gondii* of 50% for the <20 year group and 58.8% for the female 15 - 20 year group respectively. These combined results suggest that exposure to *T.gondii* is relatively common in the Waikato and that infection resulting from such exposure is not uncommon. If this is the case attention is drawn to the recent publication of concurrent infection among family members of patients with acute toxoplasmosis.[7]

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Ferritin Enzyme Immunoassay, the High Dose 'Hook Effect'.

D.H. Fallas
Laboratory, Thames Hospital

Introduction:

Ferritin is the major iron storage protein in the body. It has been concluded that Ferritin levels in patients' sera reflect iron storage status, with low serum Ferritin levels displaying high predictive value for uncomplicated iron-deficient anaemia[1]. This relationship does not always however, hold true, with elevated ferritin levels being found in inflammatory and malignant disease in the absence of adequate iron stores[1]. Furthermore, it has been found that extremely high levels of ferritin may be present on occasion, in some profound disease states[2]. These extreme elevations can demonstrate results which are anomalous to varying degrees, when assaying for ferritin by both radio-immunoassay (RIA)[3] and by enzyme-immunoassay (EIA)[2,3,4]. This is in common with a phenomenon expressed in other two site sandwich immunoassays, and is referred to as the high dose 'hook effect'. We present here one such example of this phenomenon, and show that such a result may easily be accepted as being within the reference interval, when in fact it is grossly abnormal.

Case History

A 72 year old male with a long history of previous admissions and ill-health was admitted to Thames Hospital for further assessment. His clinical condition deteriorated resulting in his death from multifactorial complications. Numerous investigations were carried out, including serum ferritin levels on more than one occasion.

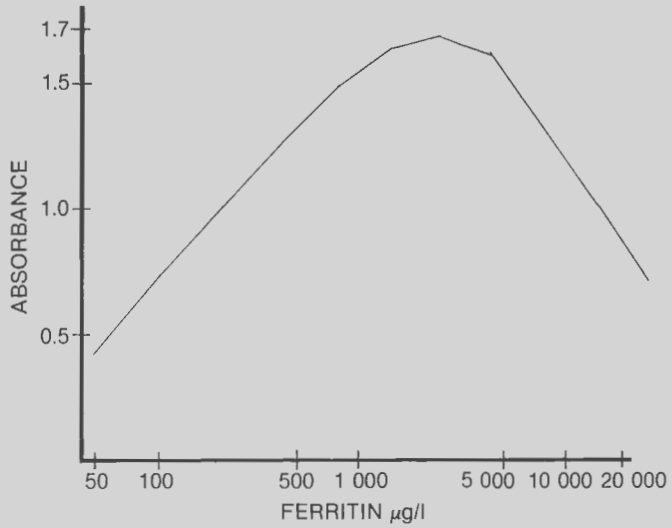
Methods

We have recently introduced an enzyme-immunoassay, Abbott Ferrizyme, Abbott Laboratories Cat.1364, into this laboratory for estimation of serum ferritin. This is a solid phase sandwich enzyme immunoassay, utilising anti-ferritin coated beads, and Horseradish peroxidase label conjugated to a second anti-ferritin. After incubation of the sample with the antibody system, there is a washing step followed in turn by colour development using o-phenylenediamine substrate with Hydrogen peroxide. Absorbance readings are measured at 492nm using an Abbott Quantum II spectrophotometer. A standard curve is automatically drawn from the absorbances of the six calibrators — calibrator values range from 0μg/l to 800μg/l. Controls and unknowns are then calculated from this curve. Samples exhibiting absorbance values greater than the highest calibrator are flagged as 'greater than 800μg/l'; repeat assay after dilution with the 0μg/l calibrator is suggested.

We detected an anomalous result when it was noted that this particular patient's serum ferritin level had apparently fallen to 129μg/l, from a value of 2400μg/l three weeks previously. The sample was re-assayed along with a twofold dilution. The value for the 'neat' serum remained unchanged, whilst the dilution gave a result of 570μg/l. This was sufficient to arouse suspicions and a further range of dilutions was subsequently performed, (2 in 3, 1 in 5, 1 in 11, 1 in 51, and 1 in 101). The final result, calculated from the 1 in 101 dilution, was 27,000μg/l. The 'hook effect' is evident when the assay response (absorbance), to the various dilutions of this sample, is plotted against nominal ferritin concentration of each dilution (Fig 1). Non parallellism in dilutions has been noted with the Abbott Ferrizyme kit[3]; this would not, however, exhibit the decreasing absorbance response to increasing concentration that we have demonstrated above 3000 to 5000μg/l.

Discussion

It is apparent that a result such as that shown above could easily have been missed, or passed over as normal, had previous assays not been performed. To help avoid this situation one could include performance of routine ten-fold dilutions on all samples. This is probably the best, but also the most expensive option. Instead, we have chosen to delete the highest calibrator (800μg/l), so that all samples exhibiting absorbance values higher than the 400μg/l), calibrator have to be diluted and re-assayed. On this



Ferritin conc. vs. Absorbance
Fig. 1

criterion alone however, we would still miss some anomalies, such as our above example. An awareness of the patients clinical condition and other laboratory results, and of the limitations of the assay itself, may further help reduce the chance of re-occurrence of similar problems in the future.

Conclusions

From our experience, we cannot support findings from a previous report[3] which found levels of greater than 27,000µg/l demonstrating 'notably high' ferritin concentrations with the Abbott Ferrizyme kit. We would suggest that falsely low levels (ie. those not notably high, and therefore not obviously requiring dilution), would be reported from samples having ferritin levels of greater than 6000 to 8000µg/l, when assayed by this particular kit. We would re-emphasise the need to exercise caution when assaying for serum ferritin in uncertain clinical circumstances, and that correlation of results to patient clinical condition and other laboratory results is of paramount importance.

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National Immunohaematology Proficiency Survey (NIPS): A Summary of Results

R.J. Austin, Charge Technologist, Blood Bank, Taranaki Base Hospital, New Plymouth and A.E. Knight, Charge Technologist, Immunohaematology Laboratory, Dunedin Public Hospital, Dunedin.

On behalf of the Technical Sub Committee of the Transfusion Advisory Committee

Introduction

This, the sixth summary of results to be presented for publication covers the last four surveys:—

NIPS 23 (November 1983)
NIPS 24 (February 1984)
NIPS 25 (May 1984)
NIPS 26 (August 1984)

These summaries are presented with no intention to pass judgement but rather for individual laboratories and technolgists to be aware of their shortcomings and to take the steps necessary to correct their deficiences.

A comprehensive summary and discussion of results is distributed to each participating laboratory after each survey which details results on a confidential basis and contains comments from the survey referees on the antibodies and/or abnormalities present. Laboratories presenting consistent deficiences are encouraged to contact their regional transfusion centre for assistance and advice.

NIPS 23 (November 1983)

(a) *Grouping*

098	A_1	Rh Positive	R_2r	K-
099	A_1B	Rh Negative	rr	K+
100	A_2	Rh Positive	R_2R_2	K-
101	O	Rh Positive	R_0r	K-

Comment — All cells were correctly ABO and Rh(D) typed by all participants. One laboratory however reported the incorrect Kell group for cell 099 and two laboratories the incorrect Rhesus genotype for cell 100.

(b) *Antibody Screening and Identification*

No atypical antibodies present. The serum 098 was however treated with Dextran to induce rouleaux formation of the test cells and some participants interpreted the results obtained as being due to the presence of an antibody.

(c) *Cross Matching*

Cell 099 incompatible due to ABO incompatibility
Cells 100 and 101 compatible

All participants carrying out the compatibility phase of the survey detected the ABO incompatibility of all techniques employed. Three laboratories did however detect incompatibilities where in fact none should have been found.

NIPS 24 (February 1984)

(a) *Grouping*

102	A_2	Rh Negative;	Kell Negative
103	O	Rh Negative;	Kell Negative
104	A_1	Rh Positive;	R_1R_1; Kell Negative
105	A_1	Rh Negative;	Kell Positive

A number of basic errors were made in the grouping section of this survey. These included transcription and transposition recording of results and highlights the care that is needed in the reading and recording of reactions.

(b) *Antibody Screening and Identification*
Serum 102 contains anti-D reactive by enzyme and Indirect Coombs techniques.
One laboratory failed to detect the presence of this clinically significant antibody by any of the techniques they employ.

(c) *Cross Match*
Cell 103 — Compatible
Cell 104 — Incompatible due to Anti-D
Cell 105 — Compatible — However this unit should not been transfused in this case due to the presence of the Kell antigen (Section 4 (3) — Blood Transfusion Procedures in New Zealand Part I).
Cell 104 was incompatible by enzyme and Indirect Coombs techniques. A small proportion of laboratories failed to detect this incompatibility by either or both of these routine techniques.

(d) *Comments*
This survey again highlighted basic errors occuring in all aspects of grouping, antibody screening and cross matching techniques. Following each survey a detailed summary is distributed to all participants. It is the responsibility of the individual laboratory to take whatever measures are necessary to bring their performance up to a level acceptable to the them.

NIPS 25 (May 1984)
(a) *Grouping*

106	A_2	Rh Positive,	R_1R_1;	Kell Negative
107	A_1	Rh Positive,	R_1R_2;	Kell Negative
108	O	Rh Positive,	R_1R_2;	Kell Negative
109	A_2	Rh Negative,	rr;	Kell Positive

Of all laboratories returning results there was 100% agreement in the ABO and Rh typing, including genotyping, of this survey. Minor errors occurred in the A subtyping and one laboratory mistyped for the Kell antigen of cell 107.

(b) *Antibody Screening and Identification*
Serum 106 contains anti-Kell plus anti-A_1.
The anti-A_1 should have been detected by all techniques employed for antibody screening and the anti-Kell readily detectable by the Indirect Coombs technique.

(c) *Cross Matching*
Cell 107 — Incompatible due to Anti-A_1
Cell 108 — Compatible
Cell 109 — Incompatible due to Anti-Kell
A number of laboratories failed to detect the incompatibles with either or both cells 107 and 109.

(d) *Comment*
Once again, this survey highlighted problems in a basic blood bank procedure namely the Indirect Coombs technique which causes the organisers some concern. It is again hoped that those laboratories and regional centres are aware of these deficiencies and have taken appropriate remedial action.

NIPS 26 (August 1984)
(a) *Grouping*

110	O	Rh Positive,	R_1R_1;	Kell Negative; $\bar{s}\bar{s}$
111	O	Rh Positive,	R_1R_1	Kell Negative; $S\bar{s}$
112	O	Rh Positive,	R_2R_2;	Kell Positive; $S\bar{s}$
113	O	Rh Negative,	rr;	Kell Negative; $\bar{s}\bar{s}$

Errors were made in the D typing, Rhesus genotyping and Ss typing phases of this survey.

(b) *Antibody Screening and Identification*
Serum 110 contains anti-$\bar{c}$ and anti-S
The anti-$\bar{c}$ was quite evident by enzyme and Indirect Coombs technique, and anti-S, as one would expect reacted by the latter technique only. A number of participants correctly detected and/or identified the anti-$\bar{c}$ with little difficulty although the anti-S caused problems for some.

(c) *Cross Match*
Some laboratories failed to detect the incompatibilities expected either by enzyme or Indirect Coombs technique or both.

(d) *Comment*
This survey elicited the lowest percentage of returns and it probably reflects the timing of the distribution coinciding with the NZIMLT conference.

General Comment
There is still a 100% participation of laboratories in the National Immunohaematology Proficiency Survey, although occasionally laboratories are failing to carry out the survey or return their results in time for including in the results summary. There are still areas of concern, the lack of suitable clerical checking procedures for the proper identification of samples and the transcription and interpretation of results, and secondly the problems some laboratories are still experiencing with enzyme techniques. These areas can only be improved by self examination of personal procedures and techniques and where necessary the assistance and advice sought from Regional Transfusion Centres. Because of the anonymity of the survey, the respective regional Charge Technologists are unable to initiate this advice. It is the responsibility of the individual laboratories to seek help and assistance.
The survey remains a popular form of external quality control and in conclusion the organisers would like, once again, to record their appreciation of all participants for their continued supprt, criticisms and supply of raw material.

Acknowledgements
The organisers wish to thank Miss Jeanette MacRea and Mrs Barbara England for their patience in interpreting and typing the surveys to date.

Is the Total Cholesterol/Albumin Ratio an Alternative to High Density Lipoprotein Cholesterol Measurement?

Jenny Shue and Paul L. Hurst*
Chemical Pathology Laboratory, Dunedin Hospital

Presented at the NZIMLT Annual Conference, Dunedin August 1984

*: Address correspondence to this author.

Running title: Albumin, cholesterol and HDL.

Abstract

A significant correlation between serum albumin and high density lipoprotein cholesterol concentration in healthy males has been reported (J Clin Pathol 1983; 36: 716-718). Based on this observation the total cholesterol/albumin ratio in serum was suggested as an alternative to high density lipoprotein cholesterol measurement as an index of risk for coronary heart disease. In seeking to confirm these findings we measured albumin, total cholesterol and high density lipoprotein cholesterol in the blood of 90 male patients admitted for coronary artery bypass graft surgery and in 125 male controls. We found no correlation between albumin and high density lipoprotein cholesterol in either group, but we did observe a significant difference in the mean total cholesterol/albumin ratios. We believe, however, that this difference is largely a consequence of the higher mean total cholesterol concentration in the patient group.

Introduction

Epidemiological and clinical studies have shown that hypercholesterolaemia is unquestionably a risk factor for coronary heart disease [1-3]. The assessment of risk in individual patients, however, is improved by also measuring the concentration (as cholesterol) of specific lipoproteins, since low density lipoprotein is atherogenic while high density lipoprotein is negatively associated with the prevalence of coronary heart disease and is considered anti-atherogenic[4,5].

With the advent of precipitation methods, the estimation of high density lipoprotein cholesterol (HDL-C) has become a routine test in clinical laboratories. In 1983, Nanji and Reddy[6] proposed the total cholesterol/albumin ratio in serum as a cheaper alternative to HDL-C measurement in the assessment of coronary heart disease risk. Their proposition was based on four observations: (a) a significant correlation between serum albumin and HDL-C concentrations in males ($r = 0.32$, $p < 0.001$), (b) a significant correlation between total cholesterol/albumin ratio and total cholesterol/HDL-C ratio in men 42-59 years ($r = 0.89$, $p < 0.001$), (c) the total cholesterol/albumin ratio separated male patients with normal or increased total cholesterol/HDL-C ratio better than total cholesterol did alone, (d) the routine availability of total cholesterol and albumin on multichannel analysers.

Analyte	Mean	SD	CV(%)
Albumin (g/L)			
Ortho normal	38.69	0.81	2.1
Ortho abnormal	29.66	0.83	2.8
Total Cholesterol (mmol/L)			
Monitrol 1	3.30	0.10	3.0
Gibcotrol high	8.84	0.18	2.0
High Density Lipoprotein Cholesterol (mmol/L)			
Ortho abnormal	0.574	0.058	10.1
Serum pool	1.587	0.050	3.2

Table 1.
Between Run Method Precision

In an attempt to confirm these findings, we measured albumin, total cholesterol (TC) and HDL-C in the blood of 90 male patients admitted for coronary artery bypass graft (CABG) surgery and in 125 male controls.

Methods

The patient group was 90 men (age range 30-73 years) who had been admitted to Dunedin Hospital for CABG surgery. Sera was obtained from blood specimens sent to the laboratory for routine pre-operative biochemical tests. The control group was 125 men (age range 17-65 years), consisting of 112 plasmapheresis/blood donors, 11 asthma drug (Sudexanox) trialists and 2 laboratory staff all of whom had been bled for routine liver function tests. Serum specimens were stored at 4 °C and analysed within 3 days.

Albumin was measured by bromocresol green dye binding on a ChemLab continuous flow analyser (ChemLab Instruments Ltd, Hornchurch, Essex, UK). Cholesterol was measured enzymatically on a Multistat microcentrifugal analyser (Instrumentation Laboratory, Lexington, MA 02173, USA) using Gilchem reagent (Gilford Diagnostics, Cleveland, OH 44135, USA). High density lipoprotein cholesterol was measured after precipitation of apo-B lipoproteins with polyethylene glycol 6000 at a final concentration of 7.5 g/L[7]. Between run precision during the six month study period (November 1983-May 1984) was monitored by Ortho (Ortho Diagnostics Inc, Raritan, NJ 08869, USA), Monitrol (American Dade, Miami, FL 33152, USA) and Gibcotrol (Gibco NZ Ltd, Penrose, Auckland) quality control sera and an in-house serum pool.

Significance of difference between means and the significance of correlation coefficients was examined by the Student's t-test[8].

Results

The analytes were measured with good precision during the six month study period (Table 1). In particular, for HDL-C and total cholesterol the SDs were within the recommendations of the Clinical Chemistry Standardization Section of the Center for Disease Control, Atlanta, GA 30333, USA, [9,10].

The data in Table 2 illustrate the well documented

	Means (SD)		
	<30 years (n = 51)	30-44 years (n = 44)	⩾ 45 years (n = 30)
Age	23.1(3.1)	36.3(4.2)	53.0(6.2)
Total Cholesterol	4.01(0.93)**	5.54(1.11)**	6.07(0.87)**
High density lipoprotein cholesterol	1.08(0.26)*	1.14(0.36)	1.11(0.35)*

* no significant difference in means between adjacent groups ($p > 0.3$)
** significant difference in means between adjacent groups ($p < 0.05$)

Table 2.
Variations of Total Cholesterol and High Density Lipoprotein Cholesterol with Age in Controls.

	Means (SD)		
	CABG (n = 90)	Controls (n = 30)	t-test p
Age	55.60(9.1)	53.00(6.2)	NS
Albumin	42.70(3.9)	44.50(2.5)	<0.02
TC	6.70(1.37)	6.07(0.87)	<0.025
HDL-C	0.98(0.25)	1.11(0.35)	<0.005
TC/HDL-C	7.71(2.45)	6.00(2.03)	<0.001
TC/Albumin	0.16(0.03)	0.14(0.02)	<0.01

TC total cholesterol
HDL-C high density lipoprotein cholesterol
NS not significant (p>0.10)

Table 3.
Comparison of CABG Patients with Controls ⩾ 45 Years

phenomena that in healthy males the mean concentration of TC increases with increasing age while mean HDL-C remains relatively constant[11]. Because of this age effect on TC it was essential that we compared the CABG group with a control group of similar age. Although the CABG patients' age ranged from 30-73 years, we chose ⩾ 45 years for our control group because 78/90 (87%) of the CABG patients were ⩾ 45 years and this division resulted in mean ages that were not significantly different between patients and controls (p>0.10). Table 3 details the results of this comparison. As we expected, mean TC and mean TC/HDL-C were higher in the CABG group while mean HDL-C was lower. Of interest was that albumin was slightly but significantly lower in the CABG patients than in controls and that the opposite was true of TC/albumin.

To determine if the lower albumin in CABG patients was related to their lower HDL-C and thus in accord with the work of Nanji and Reddy[6] we assessed the interrelationships of these parameters using the product moment correlation coefficient. The results are presented in Table 4. In both the entire control group and in the ⩾45 years control group albumin was weakly correlated with HDL-C but the value of r did not reach statistical significance. In CABG patients there was no correlation between albumin and HDL-C. The degree of correlation between TC and TC/HDL-C in all three groups was similar to the figure of Nanji and Redd (r = 0.52, p <0.001). However, when the subjects' albumin values were included in this calculation we did not observe the enhancement of the correlation coefficient reported by these investigators. Furthermore the statistical significance of the difference in the TC/albumin ratios between patients and controls was less than that seen with either HDL-C or TC/HDL-C. We believe the significant correlation between TC/albumin and TC/HDL-C seen in our results is merely a consequence of the association between TC and TC/HDL-C.

Discussion

We found no association between serum albumin and HDL-C concentrations in our population samples and thus we were unable to substantiate the work of Nanji and Reddy. Previous investigators[12-14] have looked for relationships between HDL-C and other clinical chemistry analytes and although one paper noted a negative association between albumin and low density lipoprotein cholesterol in men[14], none has reported a correlation between albumin and HDL-C. We did, however, find a lower mean albumin concentration in CABG patients than in controls but this may simply be a reflection of the generally lower albumin levels in recumbent hospitalised patients as compared with ambulant controls[14] and is probably unrelated to coronary heart disease. Moreover, we surmise that the small but significant difference in TC/albumin between patients and controls is due largely to the higher mean total cholesterol concentrations in the patient group.

Nanji and Reddy assessed the clinical usefulness of the TC/albumin ratio by its ability to classify correctly subjects with normal or increased TC/HDL-C ratios. At no stage did these authors confirm or exclude the presence of coronary artery disease in their subjects. This, we feel, is a weakness

	r	t-test, p
CABG patients (n = 90)		
Albumin vs HDL-C	0.03	NS
TC vs TC/HDL-C	0.55	<0.001
TC/albumin vs TC/HDL-C	0.45	<0.001
All controls (n = 125)		
Albumin vs HDL-C	0.13	NS
TC vs TC/HDL-C	0.53	<0.001
TC/albumin vs TC/HDL-C	0.56	<0.001
Controls ⩾ 45 years (n = 30)		
Albumin vs HDL-C	0.20	NS
TC vs TC/HDL-C	0.44	<0.05
TC/albumin vs TC/HDL-C	0.52	<0.01

HDL-C high density lipoprotein cholesterol
TC total cholesterol
NS not significant (p>0.10)

Table 4
Parameter Correlations in CABG Patients and Controls

in their investigation. Use of CABG patients in our study provided us with a population with established coronary artery disease. Although our control population was largely drawn from the local blood donor pool and thus of apparent good health, we do not claim that this group was entirely free of coronary heart disease. Despite this limitation, we believe that this paper has shown that the total cholesterol/albumin ratio is not a viable alternative to HDL-C in the assessment of risk for coronary heart disease.

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CONTINUING EDUCATION

Hereditary Non-Spherocytic Haemolytic Anaemia — A Review

B.F. Postlewaight,
Wallace Laboratory, Auckland Hospital.

The term hereditary non-spherocytic haemolytic anaemia is reserved for those subjects with a haemolytic anaemia where no major aberration of red cell morphology is demonstrable. The term congenital non-spherocytic naemolytic anaemia is often used as well. This syndrome is extremely heterogeneous both in aetiology and in its clinical manifestations. Although the syndrome is heterogeneous, there are certain common features nonetheless. Among these are an absence of appreciable spherocytosis, a normal osmotic fagility, usually no abnormal haemoglobin demonstrated and a persistence of haemolysis after splenectomy. These disorders were first described in considerable detail by Dacie et al in 1953. The disorders were classified by Dacie into type I and type II on the basis of results in an in-vitro auto-haemolysis test. When sterile blood was incubated for 48 hours at 37°C, blood from type II patients had marked auto-haemolysis which was not correctable by addition of glucose to the blood. The type II red cells were found to have impaired glucose utilisation and so a defect in glycolysis was postulated. These findings have since been confirmed and, in addition, it was found that correction of the auto-haemolysis was possible in certain instances by the addition of ATP to the blood. In these samples there was found to be a reduced level of ATP and an accumulation of certain phosphorylated glycolytic intermediates. With the advent of specific enzyme assays, the most commonly encountered cause for this metabolic clock was pyruvate kinase deficiency in the red blood cells.

The pathogenesis of these disorders remains undiagnosed in approximately three quarters of these subjects even after red cell enzyme studies and tests for unstable haemoglobins have been performed. Any disturbance in the Emden-Meyerhoff pathway may lead to premature red cell destruction as a result of defective energy production, although the precise mechanism of each enzymopathy often remains to be proven. In each case a failure of energy metabolism is the common denominator.

Within the last 30 years, recognition of distinct relationships between haemolytic disease and deficiencies of glucose-6-phosphate dehydrogenase, pyruvate kinase, glucose-phosphate isomerase, pyrimidine-5'-nucleotidase and a number of others has helped in developing an understanding of the origin of haemolytic diseases and some basic aspects of red cell metabolism. The assumption has generally been made that a cause-and-effect relationship exists between a particular enzyme deficiency and the haemolytic process being investigated. In terms of the red cell, enzyme deficiencies have now been documented which in themselves do not produce an overt haemolytic anaemia e.g. Glutathione reductase, Glutathione peroxidase and 6-phosphogluconate dehydrogenase.

Red Cell Metabolism

The mature red cell does not carry out many of the metabolic functions required for the existence of many other body cells. The red cell can not synthesise protein and is unable to further metabolise pyruvate via the citric acid cycle. The absence of these metabolic functions has not diminished the red cells' ability to survive in the circulation or to perform its major function of transporting oxygen. The principal source of energy for the red cell is glucose. Without glucose it cannot maintain the sodium and potassium gradient which exists across the red cell membrane. The red cell cannot prevent the accumulation of calcium in the red cell membrane without glucose. Methaemoglobin and oxidised glutathione will accumulate, especially when the cell is challenged by oxidated substances. The energy deprived red cell becomes morphologically altered, spheres and ultimately undergoes osmotic lysis. The red cell utilises glucose through two main routes. These are the Emden-Meyerhoff pathway and the Hexose monophosphate pathway.

In the EMP pathway a major portion of the energy so derived from glucose is stored as ATP. Reducing power is generated in the conversion of NAD^+ to NADH. The NADH reduces methaemoglobin to haemoglobin. The important modulator of haemoglobin oxygen affinity, 2,3 diphosphoglycerate (2,3 DPG) is also synthesised in this pathway.

The Hexose-monophosphate pathway accounts for approximately 10% of glucose metabolism in the red cells. In the situation of oxidative challenge, when NADPH is being oxidised to $NADP^+$, a larger proportion of the total glucose utilised may flow through this pathway. The principal function of the Hexose monophosphate pathway is to maintain NADPH in this form. The NADPH is required to maintain glutathione in the reduced form, a reaction which is important in protecting the cell from peroxidative damage.

Red Cell Enzyme Abnormalities Leading to Haematologic Disease

Enzyme	Inheritance	Morphology
Hexokinase	Autosomal recessive	Unremarkable
Glucose-phosphate Isomerase	Autosomal recessive	Unremarkable
Phosphofructo-kinase	Autosomal recessive	Unremarkable — Haemolysis and or glycogen storage disease
Aldolase	Autosomal recessive	Unremarkable — Haemolysis, mild liver glycogen storage
Triose Phosphate Isomerase	Autosomal recessive	Unremarkable — Haemolysis, severe neuromuscular disease
Phosphoglycer-ate Kinase	Sex-linked	Unremarkable — Mild behavioural changes
Diphosphoglycer-ate Mutase	Autosomal recessive	Haemolysis, polycythaemia
Pyruvate Kinase	Autosomal recessive	Unremarkable, occasional echinocytes
Glucose-6-Phosphate dehydrogenase	Sex-linked	Unremarkable, rarely "bite cells", drug, stress induced haemolysis
Glutathione reductase (complete)		Unremarkable
ɣ Glutamyl cysteine synthetase	Autosomal recessive	Haemolysis, drug or infection induced.
Glutathione synthetase	Autosomal recessive	Unremarkable — drug or infection induced
Pyrimidine-5'-Nucleotidase	Autosomal recessive	Prominent stippling
Adenosine deaminase (increased activity)	Autosomal recessive	Unremarkable
Adenylate kinase	Autosomal recessive	Haemolysis, neuro-muscular problems reported

Laboratory Diagnosis

It is generally not rewarding to attempt to establish the cause of hereditary non-spherocytic haemolytic anaemia on the basis of red cell morphology and the results of the autohaemolysis test. Red cell morphology is usually only helpful in the case of pyrimidine-5'-nucleotidase deficiency where basophilic stippling is a prominent feature. The auto-haemolysis test will not provide specific information and its use is probably restricted to the case of hereditary spherocytosis. Following splenectomy, the appearance of Heinz bodies makes the diagnosis of an unstable haemoglobin more likely. If a non-spherocytic haemolytic anaemia is suspected, it is useful to carry out screening tests initially for G-6-PD, pyruvate kinase activity and an isopropanol test for an unstable haemoglobin. If these relatively simple screening tests do not suggest a possible diagnosis, it is often appropriate to arrange for a full list of enzyme assays to be performed and further confirmation of an unstable haemoglobin be sought.

Generally, the estimation of the proportions of various red cell membrane lipids to one another and the study of membrane proteins are beyond the capabilities of all but the most specialised laboratories which are devoted to this type of investigation.

Most persons with a deficiency of Glucose-6-phosphate deyhdrogenase suffer no clinical manifestation of this common genetic trait. When they do occur, the clinical manifestation of the deficiency is a haemolytic anaemia. The effects of erythrocyte enzymopathies vary widely in humans however. Some of the enzymopathies result in haemolytic syndromes accompanied by non-haemolytic disease e.g. neuro-muscular disorders. The enzymes involved in glutathione synthesis are implicated in this group. In G-6-PD deficiency, the anaemia is usually episodic with haemolysis associated with stress, usually during drug administration, infection, diabetic acidosis and in some instances, fava beans. Some of the more unusual variants of G-6-PD may produce a non-spherocytic haemolytic anaemia.

The assay for G-6-PD activity depends on the increased absorbance at 340nm which occurs as $NADP^+$ is reduced to NADPH. The enzyme activity is related to a change in absorbance per minute and the activity is often expressed as units per gram of haemoglobin. Other enzyme assays (e.g. pyruvate kinase) are based on the conversion of NADH to NAD^+. In this case a decrease in absorbance will be measured spectrophotometrically as this conversion proceeds.

For the measurement of G-6-PD activity, a colorimetric method in which the reduction of $NADP^+$ is linked to dichlorophenol indophenol has been described. Fluorometric techniques have also been frequently used. The reduction of a number of dyes can be linked to the reduction of $NADP^+$ through NADPH diaphorases which are normally present in haemolysates. Various types of dyes have been used as the receptor dye, each with varying degrees of success, as the rate of decolorisation often varies with different lots of the same dye type.

Probably the most straightforward and reliable screening test for G-6-PD deficiency is the fluorescent spot test. This technique depends on the fact that NADPH fluoresces in long-wave ultra-violet light whereas $NADP^+$ does not. The sensitivity of the method may be improved by incorporating GSSG into the reaction mixture. When GSSG is present small amounts of NADPH which may be formed by the residual G-6-PD in mildly deficient samples are re-oxidised by the glutathione reductase reaction.

The detection of G-6-PD deficiency in males poses no difficulties. However, in females or in a patient who has recently experienced a haemolytic episode and subsequently has a large number of young cells present, problems in the detection of a G-6-PD deficiency may arise. In these circumstances, centrifugation of the blood and application of screening or assay procedures to the bottom, older layer of cells may be useful. Quantitative G-6-PD assays of red cells of such groups of patients usually indicate that activity is at the lower limits of normal in the presence of a reticulocytosis. This finding suggests that the patient may actually be deficient. Further, if the G-6-PD level is at the lower limit of normal when the activity of an age-dependent enzyme such as Hexokinase is increased, a deficiency is suspected. Family studies may also be helpful in patients in whom a deficiency is suspected.

The diagnosis of a pyruvate kinase deficiency depends on the demonstration that the activity of the enzyme is quantitatively decreased, or that well defined abnormalities are present. For assay purposes, it is particularly important to realise that the pyruvate kinase of leucocytes and platelets is not compromised in pyruvate kinase deficiency as these cells contain the genetically distinct M-type of enzyme. A haemolysate prepared from red cells which have not been thoroughly freed of contaminating white cells and platelets may give the erroneous impression that the enzyme activity is either normal or only mildly decreased. A reliable method for the removal of leucocytes and platelets is filtration of whole blood through a column containing a

mixture of microcrystalline cellulose and α cellulose.

The activity of the enzyme is measured in a coupled system. Phosphoenol pyruvate is provided in excess as substrate, and the pyruvate which is formed in the reaction serves as oxidant for NADH in the lactate dehydrogenase reaction. A decrease of PK activity is readily detected using a spectrophotometric assay procedure. It may be worthwhile to measure the activity of the enzyme at reduced levels of substrate, as more subtle abnormalities of pyruvate kinase activity may be mixed in the standard assay systems. Qualitative abnormalities can also be detected by a variety of methods utilising the assay procedure. These include heat stability tests and pH optima determination. The presence of transfused cells may have the effect of masking a pyruvate kinase deficiency.

Two screening procedures have been used for pyruvate kinase deficiency screening. One of these depends on the change in pH that occurs during the pyruvate kinase reaction and so an indicator dye is added for visual determination of enzyme activity. The fluorescent spot screening test detects the majority of deficiencies. The test reagent contains ADP, phosphoenol pyruvate as substrate and NADH. The incubated blood-reagent mixture is spotted onto filter paper and is examined for fluorescence produced by the added NADH. On incubation of the mixture, normal blood produces a loss of fluorescence as the NADH is oxidised to $NADP^+$. When a pyruvate kinase deficiency exists, the spotted filter disc continues to fluoresce.

Erythrocyte pyrimidine-5'-nucleotidase deficiency is characterised by a chronic non-spherocytic haemolytic anaemia and splenomegaly. Valentine et al demonstrated that these cells contain increased concentrations of pyrimidine-5'-nucleotides and increased glutathione levels (GSH). The basis of the screening test is an ultraviolet scan of a protein-free extract of erythrocytes. In normal cells, an absorbance peak at 258-260nm will be obtained which corresponds to that of adenine nucleotides. A sample deficient in pyrimidine-5'-nucleotidase activity shows a shifted absorbance peak at 266-270nm. These erythrocytes contain large amounts of pyrimidine-5'-ribonucleotides, namely cytidine and uridine which are derived from RNA degradation and accumulate when the enzyme catalysing their dephosphorylation is deficient. This screening test will not detect the heterozygous state for this deficiency.

In a pyrimidine-5'-nucleotidase deficiency, an increase in Heinz body formation after incubating pyrimidine-5'-nucleotidase deficient red cells with acetylphenyl hydrazine has been observed. This phenomenon is believed to be due in part to a decrease in G-6-PD activity with subsequent suppression of the pentose-phosphate shunt. The mechanism of the interference is a competitive inhibition of glucose-6-phosphate and a non-competitive inhibition of NADP for G-6-PD and 6-phosphogluconate dehydrogenase activity by a decrease in intra-erythrocytic pH brought about by the accumulation of the acidic pyrimidine-5'-nucleotides.

Haemolytic Anaemia Associated with Unstable Haemoglobin

The occurrence of haemolytic anaemia associated with the appearance of inclusion bodies was first reported in any detail in the 1940-1950 period. It was not until the 1960's that the haemolytic anaemia was firmly associated with the presence of an abnormal haemoglobin which spontaneously denatured within the red cell. These unstable haemoglobins were subsequently found to result from a mutation that changes the amino-acid sequence to one of the globin chains. A large variety of amino acid substitutions or deletions have the effect of weakening the non-covalent forces which are vital in maintaining molecular stability and if this occurs, it results in the haemoglobin denaturing and forming an insoluble precipitate. These precipitates have been shown to attach to the cell membrane proteins together with haem and with subsequently denatured membrane proteins, and are called Heinz bodies.

Functions of the Main Pathways of Glucose Metabolism in the Erythrocyte

EMP	HMP
ADP→ATP (pumps Na^+, K^+)	$NADP^+$→NADPH (reduces GSSG, protein S-G disulphides)
NAD^+→NADH (reduces methaemoglobin)	Hexose→pentose (provides substrate for nucleotide synthesis)
1,3 DPG→2,3 DPG (regulates oxygen affinity)	

Unstable haemoglobins are inherited as autosomal dominant disorders. Affected individuals are heterozygotes who have usually inherited the defect from one of their parents. Since unstable haemoglobins produce a disease state, genes for these disorders are subjected to negative selection and so the persistence of unstable haemoglobinaopathies in the population is the result of new mutations. Therefore, it is possible to encounter an individual with an unstable haemoglobin, neither of whose parents have the abnormality. The homozygous state for an unstable haemoglobin has not been encountered. The majority of unstable haemoglobins wich have been characterised have alterations in the β chain.

Laboratory Diagnosis

In the investigation of this syndrome, several features may be apparent. The haemoglobin concentration is usually decreased because of the loss of haemoglobin from the red cell as a result of its denaturation and subsequent pitting from the erythrocytes. The blood film may show slight hypochromia, poikilocytosis, polychromasia and some basophilic stippling. Reticulocytosis is often out of proportion to the severity of the anaemia, particularly when the abnormal haemoglobin has a higher oxygen affinity. Diagnosis of an unstable haemoglobinopathy depends upon the demonstration of an unstable haemoglobin. The most convenient test for this is the isopropanol test. The heat stability test should also be regarded as a useful, confirmatory procedure. The incubation of whole blood with brilliant cresyl blue generates Heinz bodies in the presence of an unstable haemoglobin. In some instances the identification or detection of the mutant haemoglobin may be aided by electrophoresis. However, in many cases the electrophoretic mobility of the mutant haemoglobin is the same as normal adult haemoglobin and so separation will not be achieved. The oxygen affinity of the unstable haemoglobin may be altered and so a P50 determination often aids in further detecting and characterising the mutant molecule. In many instances, the final diagnosis depends on globin chain separation and peptide analysis.

The interpretation of stability tests may not always be straight forward. When a particular test is being used the relative sensitivity of the test and therefore reasons for false positive results should be taken into account. The number of false positive results obtained by a particular test should be minimised without compromising too much the diagnostic value of the test i.e. its ability to detect an unstable haemoglobin.

The Heat Stability Test has been widely used and has generally been found to be reliable without being overly sensitive. In this test, washed packed cells are lysed and the haemoglobin solution is diluted in an iso-osmotic phosphate buffer, pH 7.4. The tube is then incubated at 50 °C for 3 hours. After 1 hour, 2 hours and 3 hours the tubes are examined for the presence of a flocculent precipitate. If present, the proportion of unstable haemoglobin can be determined spectrophotometrically. One point of uncertainty with this test is the choice of buffer. Investigators in this field have suggested that the use of a tris/HCl buffer makes the test more sensitive, rather than the phosphate buffer. This is because the phosphate buffer tends to have a stabilising effect on the haemoglobin

molecule by slowing the formation of methaemoglobin which normally precedes precipitation. The important feature of a tris/HCl buffer is the dependence of the pH on the temperature of the buffer solution. This is significant as the formation of methaemoglobin is favoured by a lower pH and so this temperature dependence could lead to false positive results being obtained.

Probably the most widely used test for haemoglobin stability is the isopropanol test as described by Carrell and Kay. An approximately 100g/l haemolysate is freshly prepared and added to a tube containing a 17% isopropanol solution in tris/HCl buffer, at a pH of 7.4. The tubes are incubated at 37°C. The control haemoglobin solution should remain clear for 30-40 minutes whereas the presence of an unstable haemoglobin will become apparent after 5 minutes, with the formation of a flocculent precipitate within 20 minutes. Any stability test should be observed until the commencement of precipitation of the haemoglobin from a normal control. High levels of foetal haemoglobin may give a precipitate at 20 minutes. The presence of methaemoglobin in small amounts may also give a positive result. False positive results are often due to ageing of the sample, so it is a mandatory requirement to test a freshly prepared haemolysate.

Questions: (Answers are on page 31)

1. Give two reasons why a screening test may give a normal result in the presence of a G-6-PD deficiency?
2. What is the most frequently encountered enzyme deficiency of the Emden-Meyerhoff Metabolic pathway?
3. What is the most significant abnormality noted in a stained blood film which accompanies a deficiency of pyrimidine-5'-nucleotidase activity?
4. What is the usual cause of haemolysis in a Glucose-6-phosphate dehydrogenase deficiency?
5. What is thought to be the basis for haemolysis occuring with an enzyme deficiency of the Emden-Meyerhoff Pathway?
6. What is the principal function of the Hexose monophosphate pathway?
7. What are the reasons for a falsely positive isopropanol test for haemoglobin stability?
8. Name another test which is useful in the confirmation of a positive isopropanol test.
9. Name a laboratory investigation which may be useful to perform in a case of unexplained polycythaemia.
10. Generally what is the significance of Heinz bodies in a supravitally stained preparation?
11. What is the mode of inheritance of an unstable haemoglobin?

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Revision Series in Biochemical Calculations

Section IV: pH Calculations Part B — Weak acids and Bases

Trevor A. Walmsley
Dept. of Clinical Biochemistry,
Christchurch Hospital, Christchurch.

Weak Acids

Weak acids only partially dissociate in aqueous solution to produce hydrogen ions. For example HCN, H_2CO_3, H_2S, H_2SO_3, H_3PO_4 and all organic acids are only partially dissociated in aqueous solution and are therefore weak acids.

H_2SO_4 is a special example of a weak acid that causes confusion amongst some workers and is wrongly classified as a strong acid in some text books. In fact it is only the first dissociation of H_2SO_4 that is strong and the second dissociation is weak (only 10% dissociation occurs at 0.1 mol/l). Hence the pH of 0.1 mol/l H_2SO_4 is closer to the pH of 0.1 mol/l HCl than 0.2 mol/l HCl (as some test books would have you believe).

Consider for example a 0.1 mol/l solution of monobasic acid HA. If the monobasic acid HA was a strong acid it would be completely dissociated in aqueous sclution and the concentration of hydrogen ions would be 0.1 mol/l giving a pH of 1.00 (see Section III). However if the monobasic acid is a weak acid it would only be partially dissociated in aqueous solution:—

$$H_2O + HA \rightleftarrows H_3O^+ + A^-$$

If the 0.1 mol/l solution of the weak acid HA was only 1% dissociated, the concentration of hydrogen ions would be 0.001 mol/l giving a pH of 3.00, the concentration of sale A^- would be 0.001 mol/l (since the solution **must** be electrically neutral) and the concentration of undissociated acid HA would be 0.099 mol/l.

In solution the hydrgen ion concentration can be represented either by $[H_3O^+]$ or by $[H^+]$, both are approximations used to represent the structure of a solvated proton.

The equilibrium constant K for the acid dissociation would be:

$$K = \frac{[H_3O^+][A^-]}{[HA][H_2O]}$$

Because $[H_2O]$ is effectively constant, it is more convenient to define the acid dissociation constant K_a:-

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

where
$[H^+]$ = concentration of hydrogen ions
$[A^-]$ = concentration of salt
$[HA]$ = concentration of undissociated acid
K_a = acid dissociation constant

here
$$K_a = \frac{(0.001)^2}{0.099} = 1.01 \times 10^{-5}$$

The % dissociation of a weak acid is not constant but varies with concentration — the more dilute the solution the greater is the degree of dissociation. The strength of the acid is given by its dissociation constant K_a or the corresponding pK_a where $pK_a = -\log K_a$ (c.f. pH = $-\log[H^+]$). In general the stronger the acid the larger the dissociation constant K_a and correspondingly the pK_a is smaller.

Weak Bases

Weak bases for example ammonia and organic amines are only partially dissociated in aqueous solution to produce hydroxyl ions (OH^-).

Consider for example a 0.1 mol/l solution of a weak base B, in aqueous solution this would dissociate as follows:

$$B + H_2O \rightleftarrows BH^+ OH^-$$

If the base was a strong base it would completely dissociated in aqueous solution and the concentration of hydroxyl ions would be 0.1 mol/l giving a pOH of 1.00 and a pH of 13.00 (see Section III).

However, if the 0.1 mol/l solution of the weak Base B was only 1% dissociated, the concentration of hydroxyl ions would be 0.001 mol/l giving a pOH of 3.00 and a pH of 11.00, the concentration of BH^+ would be 0.001 mol/l (since the solution **must** be electrically neutral) and the concentration of undissociated Base B would be 0.099 mol/l.

The equilibrium constant K for the base dissociation would be:

$$K = \frac{[BH^+][OH^-]}{[B][H_2O]}$$

Because $[H_2O]$ is effectively constant, it is more convenient to define the base dissociation constant K_b:-

$$K_b = \frac{[BH^+][OH^-]}{[B]}$$

where
$[OH^-]$ = concentration of hydroxyl ions
$[BH^+]$ = concentration of BH^+
$[B]$ = concentration of undissociated base
K_b = acid dissociation constant

here
$$K_b = \frac{(0.001)^2}{0.099} = 1.01 \times 10^{-5}$$

The strength of a weak base is given by its dissociation constant K_b or the corresponding pK_b where $pK_b = -\log K_b$. In general the stronger the base the larger the dissociation constant K_b and correspondingly the pK_b is smaller.

Autoprotolysis of Water

Water itself slightly dissociated because one molecule is capable of accepting a proton (hydrogen ion (H^+)) from another.

$$H_2O + H_2O \rightleftarrows H_3O^+ OH^-$$

The equilibirium constant (K) can be written as

$$K = \frac{[H_3O^+][OH^-]}{[H_2O]^2}$$

However because $[H_2O]$ is effectively constant, it is more convenient to define K_w the autoprotolysis constant of water.

$$K_w = [H^+][OH^-]$$
$$= 1 \times 10^{-14} \text{ at } 25°C$$

Relationship between K_a, K_b and K_w

Consider an aqueous solution of a weak acid HA. The acid dissociation is given by:

$$HA + H_2O \rightleftarrows H_3O^+ + A^-$$

The acid dissociation constant K_a is given by:

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad . \quad . \quad . \quad . \quad .(1)$$

Alternatively in the same solution we can consider the base dissociation:

$$A^- + H_2O \rightleftarrows OH^- + HA$$

The base dissociation constant K_b is given by:

$$K_b = \frac{[OH^-][HA]}{[A^-]} \quad . \quad . \quad . \quad . \quad (II)$$

However the relationship between the hydrogen ion concentration ($[H^+]$) and hydroxyl ion concentration ($[OH^-]$) is given by K_w where

$$K_w = [H^+][OH^-]$$

From equation I

$$[H^+] = \frac{K_a [HA]}{[A^-]}$$

and from equation II

$$[OH^-] = \frac{K_b [A^-]}{[HA]}$$

therefore

$$K_w = \frac{K_a [HA]}{[A^-]} \times \frac{K_b [A^-]}{[HA]}$$
$$K_w = K_a \times K_b$$
$$= 1 \times 10^{-14}$$

taking logs

$$\log K_w = \log K_a + \log K_b$$
$$-\log K_w = -\log K_a - \log K_b$$
$$pK_w = pK_a + pK_b$$
$$= 14$$

Example:— Calculate the pH of 0.1 M acetic acid

Given that $K_a = 1.82 \times 10^5$

Acetic acid dissociates in aqueous solution:—

$$HAc \rightleftarrows H^+ + Ac^-$$

therefore

$$K_a = \frac{[H^+][Ac^-]}{[HAc]}$$

where [HAc] = concentration of acetic acid (mol/l) = (0.1 M -$[H^+]$) — this approximates to 0.1 M in this example since $[H^+]$ is relatively small due to the low % dissociation. Where the % dissociation is > 10% this approximation is not valid (see example on sulphuric acid).

$[H^+]$ = hydrogen ion concentration (mol/l)

$[Ac^-]$ = concentration of salt (mol/l) = $[H^+]$ since the solution must be electrically neutral.

therefore
$$K_a = \frac{[H^+]^2}{[HAc]}$$
$$[H^+]^2 = K_a \times [HAc]$$
$$= 1.82 \times 10^{-5} \times 0.1$$

therefore
$$[H^+] = \sqrt{1.82 \times 10^{-6}}$$
$$= 1.35 \times 10^{-3} \text{ mol/l}$$
$$pH = -\log (1.35 \times 10^{-3})$$
$$= 2.87$$

To calculate the % dissociation of HAc

$$\% \text{ dissociation} = \frac{[H^+] \times 100\%}{[HAc]}$$
$$= \frac{1.35 \times 10^{-3} \times 100\%}{0.1}$$
$$= 1.35\%$$

Example:— Compare the pH of 0.1 M HCl and 0.1 M H{SO|:-

HCl is completely dissociated in aqueous solution:—

$$HCl \rightarrow H^+ + Cl^-$$

Therefore the concentration of hydrogen ions in solution is 0.1 mol/l and the pH is 1.00.

If complete dissociation of 0.1 M H_2SO_4 occurred the concentration of hydrogen ions would be 0.2 mol/l and the pH of 0.1 M H_2SO_4 would be 0.70. (equal to the pH of 0.2 mol/l HCl).

However although the first hydrogen ion completely dissociates in aqueous solution:—

$$H_2SO_4 \rightarrow H^+ + HSO_4^-$$

The second hydrogen ion only partially dissociates:—

$$HSO_4^- \rightleftarrows H^+ + HSO_4^-$$
$$K_{a^2} = 1.2 \times 10^{-2}$$

therefore

$$K_{a^2} = \frac{[H^+][SO_4^-]}{[HSO_4^-]}$$

where $[H^+]$ = hydrogen ion concentration

= 0.1 mol/l from the first dissociation + x mol/l from the second dissociation

$[SO_4^-]$ = x mol/l

$[HSO_4^-]$ = 0.1 mol/l - x mol/l

here
$$1.2 \times 10^{-2} = \frac{(0.1 + x)\,x}{0.1 - x}$$
$$= \frac{x^2 + 0.1x}{0.1 - x}$$

$$0 = x^2 + 0.112x - 1.2 \times 10^{-3}$$

compare with $0 = ax^2 + bx + c$ which is the general formula for a quadratic equation where

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

therefore
$$x = \frac{-0.112 \pm \sqrt{0.01254 + 4.8 \times 10^{-3}}}{2}$$

$$= \frac{-0.122 \pm 0.1316}{2}$$

$$= 0.0098 \text{ mol/l}$$

$$\% \text{ 2nd dissociation} = \frac{[H^+] \times 100\%}{[HSO_4^-]}$$

$$= \frac{0.0098 \times 100}{0.1}$$

$$= 9.8\%$$

therefore $[H^+] = 0.1 + 0.0098$

$= 0.1098$ mol/l

$pH = -\log 0.1098$

$= 0.96$

Hence the second dissociation of H_2SO_4 is only 9.8% and therefore the pH of 0.1 M H_2SO_4 is much closer to 0.1 M HCl than 0.2 M HCl.

Salts of Weak Acids

Salts of weak acids undergo hydrolysis in solution to produce an alkaline solution.

Example:— Calculate the pH of 0.1 M Sodium Acetate (NaAc) given that the K_a for HAc = 1.82×10^{-5}

Sodium Acetate is completely dissociated in solution:-

$$NaAc \rightarrow Na^+ + Ac^-$$

The acetate ion associates with water to produce hydroxyl ions:—

$$Ac^- + H_2O \rightleftharpoons HAc + OH^-$$

therefore $K_b = \frac{[HAc]\,[OH^-]}{[A^-]}$

where K_b = base dissociation contant for acetate ions

$$= \frac{K_w}{K_a}$$

$$= \frac{1 \times 10^{-14}}{1.82 \times 10^{-5}}$$

$= 5.49 \times 10^{-10}$

$[HAc]$ = acetic acid concentration

$= [OH^-]$

$[A^-]$ = acetate ion concentration

$= [NaAc] - [OH^-]$

= approximately 0.1 mol/l

therefore $[OH^-]^2 = K_b \times [A^-]$

$= 5.49 \times 10^{-10} \times 0.1$

$[OH^-] = \sqrt{5.49 \times 10^{-11}}$

$= 7.41 \times 10^{-6}$

$pOH = -\log [OH^-]$

$= -\log 7.41 \times 10^{-6}$

$= 5.13$

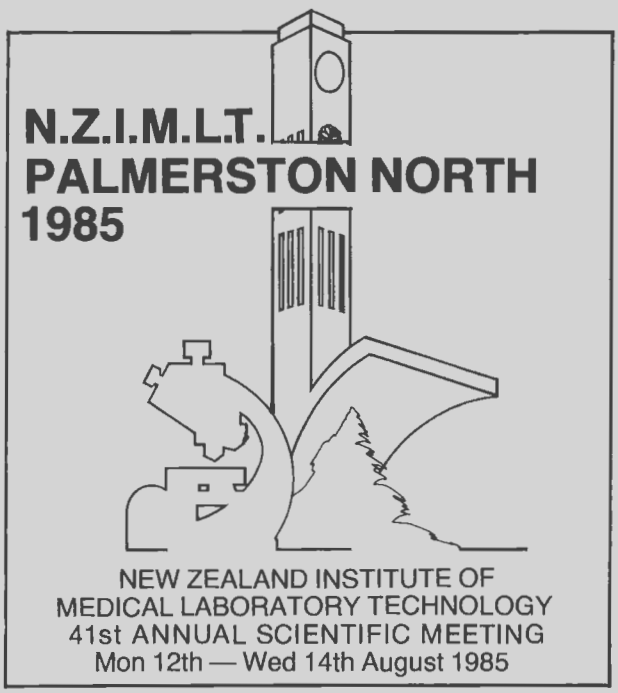
therefore $pH = 8.87$

Salts of Weak Bases

Salts of weak bases undergo hydrolysis in solution to produce acidic solutions. For example a solution of ammonium chloride NH_4Cl is acidic.

Problems (Answers on page 31)

1. Given the following dissociation constants:—
   a) Acetic Acid ($CH_3.COOH$) $K_a = 1.82 \times 10^{-5}$
   b) Monochloracetic Acid ($CH_2Cl.COOH$) $K_a = 1.40 \times 10^{-3}$
   c) Dichloroacetic Acid ($CHCl_2.COOH$) $K_a = 3.32 \times 10^{-2}$
   d) Trichloroacetic Acid ($CCl_3.COOH$) $K_a = 2.00 \times 10^{-1}$
   Calculate the corresponding pK_a values.
   Which is the strongest acid?
2. Given the following pK_a values:-
   a) Ammonia (NH_3) $pK_a = 9.25$
   b) Methylamine ($CH_3.NH_2$) $pK_a = 10.6$
   c) Dimethylamine ($(CH_3)_2.NH$) $pK_a = 10.7$
   d) Trimethylamine ($(CH_3)_3.N$) $pK_a = 9.81$
   Calculate the corresponding base dissociation constants.
   Which is the strongest base?
3. Calculate the pH and % dissociation of (a) 0.1 M, (b) 0.05 M, (c) 0.01 M, (d) 0.005 M and (e) 0.001 M solutions of acetic acid. Does the % dissociation increase or decrease with concentration? ($pK_a = 4.74$).
4. Calculate the pH of 0.1 M ammonium chloride (NH_4Cl) given that pK_b $NH_4OH = 4.75$.
5. Given that 100 ml of a 0.1 mol/l solution of a weak monobasic acid has a pH of 3.00 what would be the pH of the solution if it was diluted to 1000 ml with distilled water. Compare this with a solution of HCl of pH 3.00 that is similarly diluted.



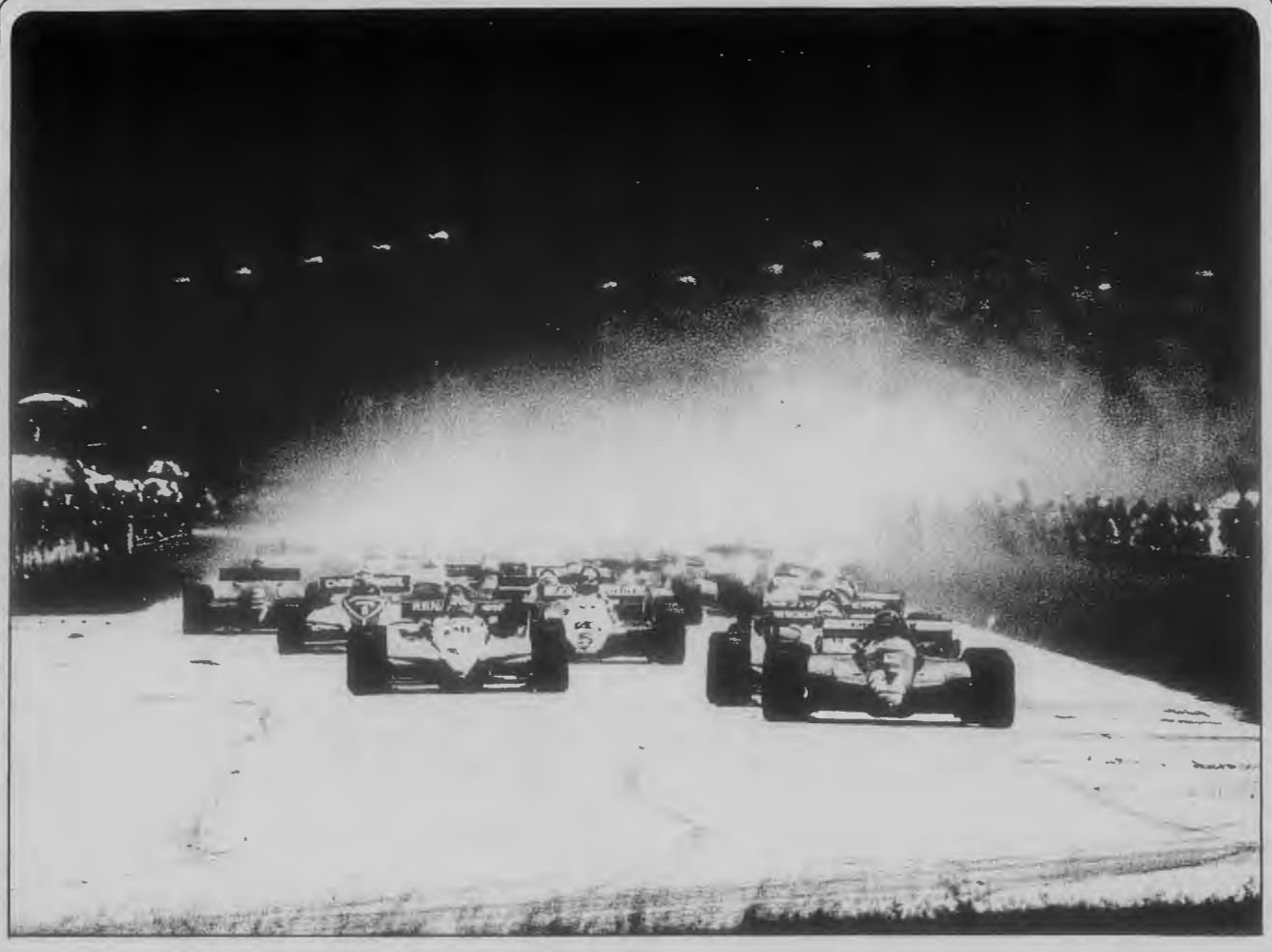
Theme: "Towards the Year 2000"

In view of the speculative nature of the theme the organisers have decided to depart from the established procedure of submitted papers, and aim to have a structured forum with invited speakers.

It is anticipated that this will be of general interest to all technologists and active participation is encouraged in the form of discussion groups, seminars and workshops, by all delegates.

Currently it is proposed that there could be two concurrent workshops in microcomputers — Basic and Advanced, and a workshop on "Semi-automated ABO and RH typing". Any ideas for further workshops from members is encouraged, and they may be accommodated if sufficient interest is revealed.

Any such ideas should be submitted before early April 1985, (along with the screams of outrage) to Dave Hepden, Conference Secretary, Haematology Department, Palmerston North Hospital, Private Bag, Palmerston North."



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Applications will be judged on your professional and academic abilities, together with the service you have given to both the profession of Medical Laboratory Technology and/or the NZIMLT. The definition of service is broad and incorporates the activities of those who have been willing to act as examiners, tutors, organisers of local branches, or contributed to the improved understanding of the science of clinical pathology.

Applications/Nominations must be on the official form and received by the Secretary, NZIMLT no later than 5 p.m., 28th June 1985. Late applications or nominations will not be accepted.

The decision as ratified by the council of the NZIMLT will be final.

CLOSING DATE JUNE 28th 1985

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(Strike out that which is not applicable)

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(Applicant's name or person being nominated)

ADDRESS: .....

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I agree to abide by the terms of the Award and the decision of the judges.

SIGNED: Nominee / Applicant ..... DATE: .....

(Delete as appropriate)

This application form must be accompanied by references from:

(a) The Director or Senior Medical Officer in charge of your laboratory.

(b) Any other unrelated individual.

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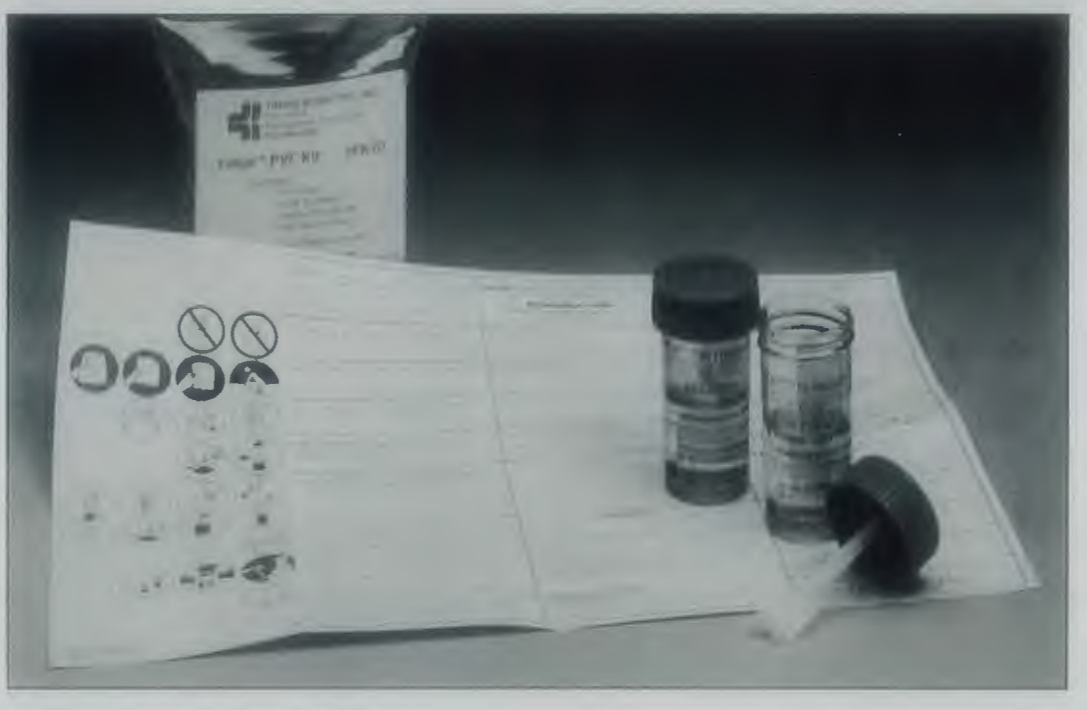

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Answers to Section IV: pH Calculations Part B

1. a) $pK_a = 4.74$
   b) $pK_a = 2.85$
   c) $pK_a = 1.48$
   d) $pK_a = 0.70$ — strongest acid

2. a) $K_b = 1.78 \times 10^{-5}$
   b) $K_b = 3.98 \times 10^{-4}$
   c) $K_b = 5.01 \times 10^{-4}$ — strongest base
   d) $K_b = 6.46 \times 10^{-5}$

3. a) pH = 2.87 dissociation = 1.35%
   b) pH = 3.02 dissociation = 1.91%
   c) pH = 3.37 dissociation = 4.27%
   d) pH = 3.52 dissociation = 6.04%
   e) pH = 3.87 dissociation = 13.5%
   As expected the % dissociation increases with dilution.

4. pH = 5.13

5. pH of weak monobasic acid after dilution = 3.50
   pH of HCl after dilution = 4.00

Answers to questions on Hereditary Non-Spherocytic Haemolytic Anaemia

1. a) Presence of large numbers of reticulocytes
   b) Presence of transfused cells
2. Pyruvate kinase deficiency
3. Prominent basophilic stippling
4. Oxidative stress
5. ATP lack whereby the cells are unable to maintain their many functions
6. To maintain $NADP^+$ in its reduced form
7. a) Testing an aged sample — presence of methaemoglobin
   b) High levels of foetal haemoglobin in sample
8. Heat stability test
9. Oxygen affinity studies
10. a) Presence of an unstable haemoglobin
    b) Precipitated haemoglobin as a result of oxidative stress
11. Autosomal dominant

Obituary

Mrs Audrey Alexandra North (nee Prentice)

Audrey, who trained as a Medical Laboratory Technologist and qualified COP in 1944 died October 31, 1984.

Audrey's training commenced in 1939 at Christchurch Hospital and after qualifying COP she raised a family of five children. In 1965, Audrey returned to work in the Nelson Hospital Laboratory taking charge of the Transfusion and Donor Services until the family moved to Auckland. Audrey worked from 1979 until six weeks prior to her death, when ill health prevented her working in the Tissue Typing Dept., Auckland Blood Transfusion Centre. Audrey orchestrated the programme of Renal patients awaiting kidney transplants and accepted the challenge of managing their records on computer files.

She was always a very active and willing worker, who put aside her pain and health problems of the last few months, usually as "More of a nuisance than anything" so that she continued working.

Audrey will be missed by us in the department. We can remember her dedication to work as a Medical Technologist and her courage and cheerfulness through illness as an inspiration to all her co-workers.

LABSUPPLY PIERCE

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Your suggestions for future courses:

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The Pacific Way

Laboratory Services on Norfolk Island

Anne Morpeth

Norfolk Island is a small island (approximately 5km by 10km) situated about midway between New Zealand and New Caledonia, with a population of approximately 1,800. It is a beautiful, peaceful island away from the rush and pressures of modern city life with a fascinating, if brutal, history — it was a penal colony in the 18th and 19th centuries. The "Islanders" are descendents of Pitcairn Islanders who were originally the mutineers of the Bounty. They are a friendly, easy-going people with their own language — a lilting melodic, pleasant sounding language that is a sort of mixture of Tahitian and Olde English. There are no great extremes of wealth apart from several millionaires and certainly not the poverty seen in some Pacific Islands.

The hospital has 21 beds and an operating theatre, intensive care unit, maternity wing etc. There are two doctors employed by the Government for a two year term and they consult from the hospital. There is no private practice as such, although one of the residents is a Dermatologist, and she consults part-time at the hospital. In the hospital there are about five geriatric ladies who are permanent in-patients, and they bring the bed occupancy to an average of about ten. This number is increased while visiting specialists are here, particularly surgeons. During the year we usually have a Gynaecologist, Orthopaedic Surgeon, ENT Surgeon, General Surgeon, Opthalmologist and Paediatrician visiting.

The doctors see about 45 patients a day and this is where the majority of the routine work for the laboratory comes from. The local population is generally fairly healthy and the common problems such as hyperlipoproteinaemia and hyperuricaemia probably result from a high standard of living. Most of the major illnesses seem to occur in elderly tourists.

The laboratory first started about 1977. It was staffed by the Matron and could only do haemoglobins, ESR's and glucoses, using a small Unimeter. Someone suggested that the Unimeter would give more stable results if it was left on all the time. This was tried but unfortunately it caught fire, doing a small amount of damage to the hospital. The Lions Club came to the rescue buying a new spectrophotometer but there was nobody who could operate it to its full capacity. The range of tests at this time, though, was increased to include ureas and cardiac enzymes. Fortunately, in 1979 a laboratory technologist arrived on the Island with her husband. He was here on a two year term with the Meteorological office and she offered to work part-time. She was able to do basic haematology and biochemistry tests and had to send everything else to a laboratory in Sydney. When she left, after her husband's term was up, the laboratory was again staffed by a nursing sister. The doctors found this unacceptable and pushed for a full-time trained technologist. This was towards the end of 1981 and I was accepted for the position. At the time I was working as a staff technologist in the Biochemistry Department at Middlemore Hospital where I had trained and worked for nearly ten years.

I was absolutely amazed at the size of the laboratory (not much bigger than a broom cupboard) and at the antiquity of the equipment; the microscope had been left by one of the Doctors who finished his term here in 1936. Apart from that there was only the spectrophometer, a centrifuge, incubator and blood-bank refrigerator. There was a general lack of basic laboratory apparatus such as pipettes, test-tubes etc. No controls were used and most of the reagents were out of date. It came as quite a shock and took me right back to the basics of laboratory technology after being conditioned to the sophistication of a large modern hospital laboratory.

Anne Morpeth

As time went by I set up a microbiology section and increased the number of tests to cover quite a comprehensive range. Finance is always a problem in a small community. The Island is a semi-autonomous Territory of the Commonwealth of Australia but the Australian Government does not give any assistance to the Hospital. The Hospital Board is given a modest subsidy by the Local Government and subsequently relies very heavily on the Island's service organisations. They have donated such things as the Ambulance, hospital beds, Baby Clinic, Intensive Care Unit, etc. The laboratory has been greatly assisted by the Lions Club, and through this organisation has recently acquired a new Olympus microscope and two Ames glucometers. In turn, I have helped the Lion's Club with a diabetes survey of the Island. I was assisted by the Matron and two nursing sisters and between us we did blood sugars and blood pressures on 773 residents in a day and a half. We must have covered just about the entire adult population and it was an interesting and worthwhile exercise, although very hard work. We picked up two definite diabetics, a number of borderlines and quite a few hypertensives.

The most recent acquisition by the laboratory is a new spectrophotometer with a small microprocessor. It is far superior to the old one and should enable me to expand the range of tests even further. Next on the list, I hope, is a new and bigger laboratory!

Although it is only a small hospital catering for a small population, it is necessary to do a wide range of tests because of the Island's geographical isolation. Results take three to four weeks to get back from Sydney (if they don't get lost) and freight and courier charges make it very expensive to send tests away. On the other hand it can be very costly doing tests individually making it necessary to batch routine work as effectively as possible. Another difficulty is that of supplies. It is essential to order consumables well in advance and even then they can take months to arrive. Often the distributor will forget to write "Air Mail" on goods asked for by air parcel post and they will arrive a couple of months later on the boat. Or else airfreight will be backlogged for up to eight weeks as the planes are full and there is no room for freight. It is expensive making toll calls and sending telexes trying to locate lost freight. One cannot just phone a neighbouring laboratory and borrow some reagent until supplies arrive. Also it is very difficult to find out exactly what is available from the different companies, and what advances have been made in the field of medical laboratory technology.

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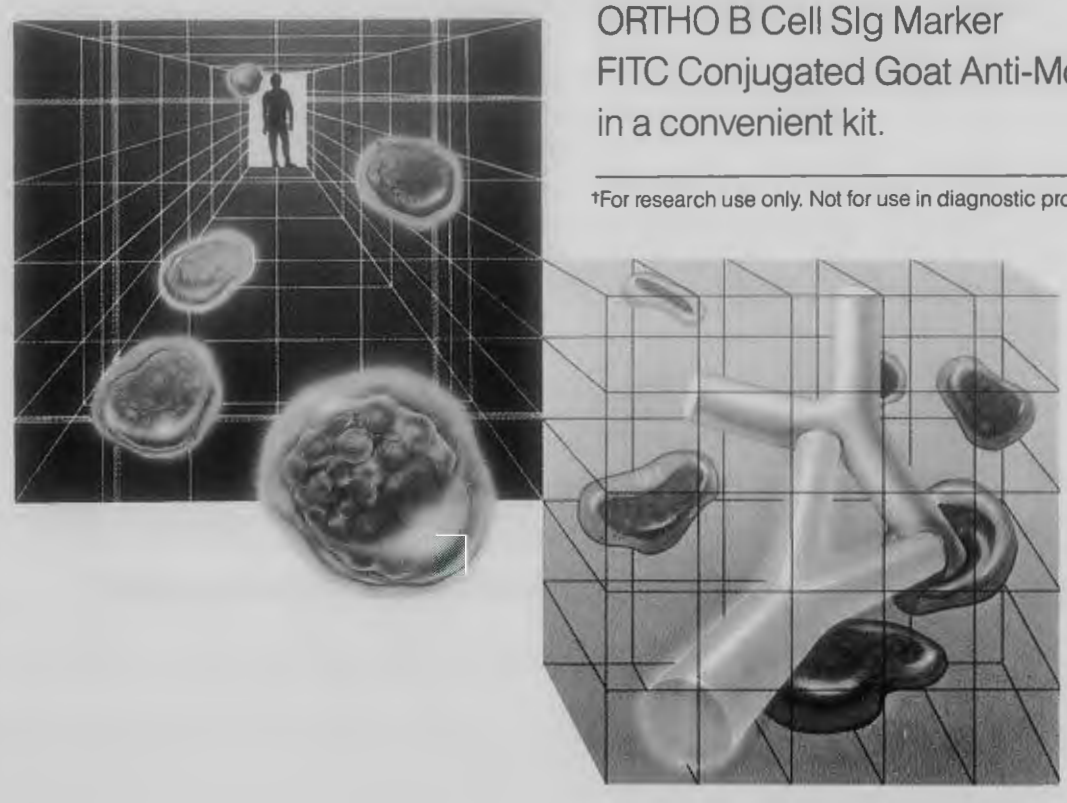

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different areas usually covered in private and hospital laboratories. I have been called upon to do some very strange things, many of which I know nothing about. For example, recently I was asked to identify mould growing in a can of beer and also to find a cause for the decimation of a local grower's tomato crop. Just prior to that I had to assist the Medical Superintendent in performing a post mortem on a green parrot, taking swabs and making blood films. Neither of us really knew what we were doing or what we were looking for. However the problem is that these sorts of things cannot be sent into Australia or New Zealand due to agricultural regulations. I also act as the local veterinary laboratory, test bore water for salinity as well as help the health inspector from time to time on water testing and food poisoning cases.

Once a month we receive six units of packed cells from Australia and in return send them six units of whole blood from local donors. They screen and type these fully, use them then send us the donor cards so that we have on hand a good selection of types and screened donors in case of emergency. The local branch of the Red Cross Society assist and provide tea and biscuits while I take the blood. It is always an amusing, if busy, afternoon at the hospital. One of the tremendous advantages of the job is the close communication with doctors, nursing staff and patients. This enables one to follow a case from all aspects and the doctors go to a great deal of trouble explaining the clinical side of the cases, discussing x-rays, ECGs etc. The patient becomes more than a number on a test-tube.

The major disadvantage is being the sole laboratory technologist on the island. If I am unsure of something there is no-one to turn to and say "come and have a look at this and tell me what you think". Also if I am sick I still have to front up if I am needed. There has been the odd occasion when I have felt that the patient was in better condition than me. I am 'on call" 24 hours a day, seven days a week although I am not called in that often except perhaps on weekends. I cannot just take a few days off if I want to. The workload, approximately 500 procedures a month, doesn't warrant any additional staff but it would be great if there was someone, perhaps retired or with a family, that could fill in occasionally when required.

Norfolk Island is a lovely place to live — warm climate, nice beaches, clean air and plenty of recreation facilities. It is an easy, slow way of life with no queues, crowds or traffic jams — the speed limit is 50km/hour and the cows have absolute right of way. Anybody here on holiday is welcome to call in to the hospital at any time.

LETTERS TO THE EDITOR

Dear Sir,

RE: Use of I or L

A recent publication making a recommendation on the abbreviations for the quantity — litre has been brought to my attention. The publication the International Standards Organisation (ISO 1000) reads:—

> "...optional symbols are given for indicating the litre (I and L). While it has been past practice to use I as the symbol for the litre and this symbol will be found in earlier British Standards. L is now preferred, and should be substituted when the symbol for the litre occurs in the subsequent text. This avoids possible confusion between the arabic numeral '1' and the lower case letter 'l'".

It is my recommendation that the same system be used throughout New Zealand Laboratories and therefore the Upper Case L should be used as the symbol for the quantity litre.

I would appreciate it if this could be published in your journal.

Yours sincerely,
C.W. Small
CHAIRMAN/SECRETARY
JOINT COMMITTEE on SI UNITS

Pay Rates

Dear Sir,

I hold the senior technologist's position in the hospital's Biochemistry department, designated level 2 of the Grade laboratory officer scale.

With my family of three children and my present position on the salary scale, I qualify to receive help under the Family Care scheme for low income families.

I look forward to hearing the results of the Negotiations Committee activities.

Yours sincerely,
Steve Joyce Christchurch

A.I.M.L.S. National Scientific Meeting, Hobart 14th-16th August 1985

The National Scientific Meeting of the A.I.M.L.S. is being held in Hobart on the 14th-16th August 1985. There has been a lot of early interest shown in the meeting and several overseas speaker have already been booked to attend. At this stage we are calling for proffered papers in all disciplines and abstract forms will be supplied to any one interested.

Registration forms will be available from the Secretary, NZIMLT, Haem Dept, Christchurch Hospital, Christchurch or from the Conference Secretary from early March.

The direct air link between Christchurch and Hobart along with discount advanced paid fares make for an attractive proposition. T.A.A., official carrier to the meeting, will be pleased to discuss these matters via their Christchurch office and will also be able to supply details of many pre and post meeting Tasmanian tours that are available.

Address for correspondence:
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Tasmania 7250

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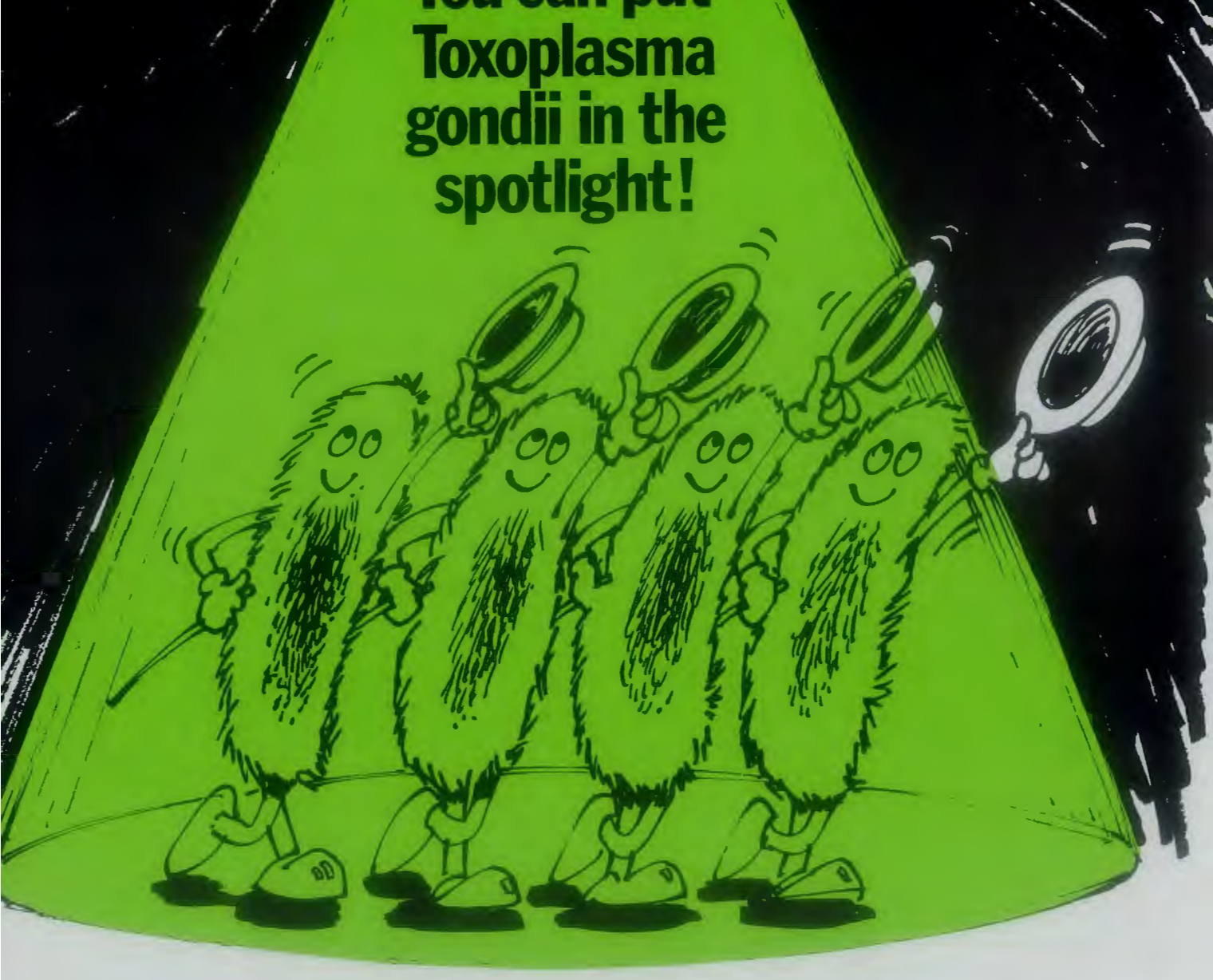

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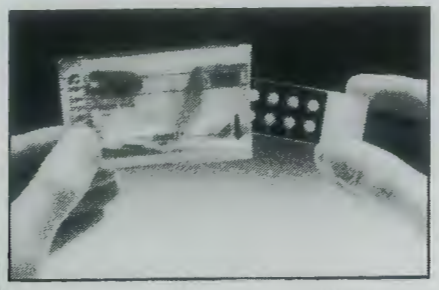

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Membership Fees and Enquiries

Membership fees for the year beginning April 1, 1985 are:
For Fellows — $45

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For Members — $30

For Non-practising Members — $20

All membership fees, changes of address or particulars, applications for membership or changes in status should be sent to the Membership Secretary at the address given above.

Members wishing to receive their publications by airmail should contact the Editor to make the necessary arrangement.

Senior Members Honoured

At a recent meeting of the Council of the NZIMLT, Joan Byres and Ian Cole, both of Auckland, were elected Honorary Members of the NZIMLT. Our congratulations to them on receiving this honour in recognition of their services to the profession.

Joan Byres

She commenced medical laboratory technology in 1937 at Auckland Hospital. Joan qualified then travelled to England where she worked with Daisie McFarlane at Radcliffe Infirmary and also at Hammersmith Postgraduate Medical School. On returning to Auckland Hospital, Joan became the Haematology Charge Technologist. Then in 1945 moved to Greenlane Hospital laboratory as Charge Technologist; Dr Gilmore was the Senior Pathologist with Drs Cairns and Doyle. During this time she was on the committee of the NZ Bacteriologists Association (now the NZIMLT). In 1949 Joan joined the private pathology practice of Dr Fowler which evolved into Diagnostic Laboratory. In recent years Joan has been the laboratory Purchasing Officer.

Ian Cole

Qualified C.O.P. in 1949 after training at Auckland Hospital. Ian was placed in charge of various departments before being appointed Charge Technologist at Middlemore Hospital Laboratory. In 1952, Ian was appointed Charge Technologist at Greenlane Hospital with approximately 6 staff under him. He developed many methods specifically for the laboratory requirements at Greenlane Hospital such as techniques for the sterilization of heart valves. Ian served as Junior member of Council and on various sub-committees over the years as well as many committees for the Auckland Hospital Board, in particular those on laboratory education and the Marion Davis Library. He wrote papers on many aspects of laboratory procedures including laboratory management and safety. In 1981, Ian was presented with an award for outstanding service to Greenlane Hospital. He retired in 1984 as Senior Principal Technologist to the Auckland Hospital Board. Ian is now able to devote time to his other interests — art, antiques, gardening, freemasonry and his passion for cricket; he has acted as cricket administrator on many overseas tours.

Report to the NZIMLT of the Meeting with the Health Service Personnel Commission held in Wellington on 16th October 1984.

The NZIMLT was represented by John Elliot, Colvin Campbell and Walter Wilson and the meeting was attended by approximately 10 other health employee organisations and a representative from the Ombudsman's Office.

The meeting was chaired by Mr Henry Smith, Chief Executive Health Service Personnel Commission who opened with a discussion on the new procedures for setting and reviewing gradings.

Section 26 — The establishment and Review of Graded Positions.

1) The commission staff will set grades for new positions.
2) Reconsideration of any established positions may be requested by submission to the Commission by a Hospital Board at any time throughout the year. The submissions are to be in narrative form and not necessarily restricted to any particular proforma design.
3) If the Hospital Boards do not receive a favourable result they may resubmit the request as many times as they consider necessary or appropriate until they receive a satisfactory reply.
4) The emphasis is on grading positions and not individuals and he considers the Commission staff is better equipped to undertake this task than a committee such as our old grading review committee and that to include employee organisations at this stage would be inappropriate.
5) By not having an annual reminder perhaps abuse of the rights of review might be lessened; also it will relieve the workload during April to July produced by the old annual review.
6) Employee organisations can inform the Commission of anomalies they consider exist in a particular establishment, hospital board or nationally without prior reference or consultation with the Hospital Board's in the hope of gaining a review. The commission will make available upon request all grading structures.

Function and composition of Technical Committees.

1) These are to be ad-hoc and not Standing Committees.
   Members will be chosen by Commission staff who will not normally consult the employee organisation but will probably appoint Health Service staff from the occupational group under review.
2) These Technical Committees will be used when it is considered necessary to review a number of graded positions; a general review of an occupational class or a review of grading steps or procedures.
3) Broad banding is out in principle and if specific problems exist then a general review of the occupational group's grading structure will be undertaken to attempt to produce a more appropriate structure.

Section 27 — Individual Rights for Review of Grading

1) Because of the new Act everybody can as of the 1st April seek a personal reconsideration.
2) Personal reconsiderations are to be sent directly to the Commission and not necessarily through the employing Hospital Board but it is probably good practice and common courtesy to inform the Board of the application. The Commission is required to consult with the Board before making any decision.
3) It is not clear whether the 5 year interval is for a person in a particular position or a person irrespective of the position e.g. if they change to a new job having recently had a personal appeal they might not be eligible for another personal appeal until the 5 year period has elapsed.
4) The Commission will disclose its reasons for rulings at all stages and not only when required under the Act.
5) People making applications to the Grading Review Committee or the Appeal Board must consider the use of practiced counsel as the Commission will be using practiced counsel. The commissions policy will be to make a particular Commission staff member responsible for a decision right from the initial application through to the Appeal Board if the claim is pursued that far.
6) It must be emphasised to all Health Service employees the need to present a sound and full case in any claim for regrading.

General

1) The Commission being the ultimate employing authority will make a final decision on any matters referred to it.
2) Any Health Service employee can individually petition the Commission if not satisfied with a Hospital Board's ruling. This will not be promoted by the Commission as it prefers any such request to be channelled through the appropriate employee organisation.
3) He emphasised that the Commission is independent of the Health Department and Hospital Boards and it has the statutory responsibility for the maintenance of appropriate conditions and salaries etc for Health Service employees and therefore their decisions will not necessarily be influenced by the financial situations of individual Hospital Boards and to some extent Health Department policy. He emphasised that this will be the policy particularly when reviewing individual applications and clearly if an applicant meets the workload and responsibility criteria etc of a higher graded position then it will be encumbent on the Board concerned to pay the higher grade or change the workload etc.

Conclusions

While Mr Smith expressed a desire for co-operation and improvement in relations it was to be realised that virtually all of his staff are people who have transferred from the Health Department and indeed will be familiar faces to all of those involved in negotiations and dealings with the department.

However, because of its statutory existence and whereas in the past the State Services Commission technically had the same responsibility but apparently did not wish to exercise their rights, it may be easier to enforce negotiated decisions through the Health Commission than has been the past practice.

Subsequent to the meeting with the Commission an informal meeting was called by the employee groups present at the meeting to discuss common practice and policy.

One of the principal points of concern was that by submitting each claim in complete isolation without regard or discussion with any other group we are playing into the hands of the employing authority who can then play one group against the other to their advantage and it was felt we will have to put aside many of our suspicions, parochialism, sceptism etc and generally co-operate much closer with each other prior to the lodging of claims so that on those issues where we have a common interest we can present a united front.

The nurse's representative also advised the meeting that he had recently recirculated all of the employee groups regarding the DG48 committee to seek their advice as to whether they wish this committee to continue to function and indeed it was intimated by Henry Smith that the Commission would welcome a united approach for negotiations of DG48 and would do all that they could to see that such an informal group be legally recognised.

W.J. Wilson
Vice President

NEGOTIATIONS

The Negotiations Committee of the NZIMLT lodged a salary claim on behalf of medical laboratory workers with the Health Service Personnel Commission late last year. Printed below is the claim and then a report on the response.

The Claim

Dear Sir,

The following salary claim is submitted on behalf of the NZIMLT. For your information the members of the Negotiations Committee are —

Mr B.W. Main	Dunedin
Mr C. Campbell	Palmerston North.
Mr W. Wilson	Auckland
Mr J. Elliot	Wellington
Mr P. McLeod	Nelson

(1) Extensive revision of the wording of H.S.19 is required to bring it up to date. For this purpose the current salaries at 10.11.81 have been left out in order to reduce bulk. Changes to the wording are highlighted in pink, deletions will be obvious by reference to the present printing or by bracketed comment. Many of the changes are brought about by the introduction of the Health Service Personnel Commission as the employing authority and its role in the grading of positions. Others relate to changes in the Medical Laboratory Technologists' Board regulations of 1982. The final draft will of necessity have to await the outcome of negotiations on the salary claim. The revised H.S.19 is appended as a separate schedule.

(2) **Salaries**

(a) *Graduate Technologist Scale*

The basic rates for this scale are Grades I and II of H.S.51 Scientific Officers. We have ascertained that there are still four people on this scale and they should be protected by bringing the scale into line with H.S.51. Agreement was reached between us in 1981 on the following scales. It is essential that it be reiterated that no new appointments be made to the scale after **1978.**

	10.11.81	+ C.O.L. Allowance	
(i)	22,340	(ii)	17,001
	20,969		16,342
	19,643		15,672
	18,320		15,074
	17,660		14,489
			14,046
			13,434

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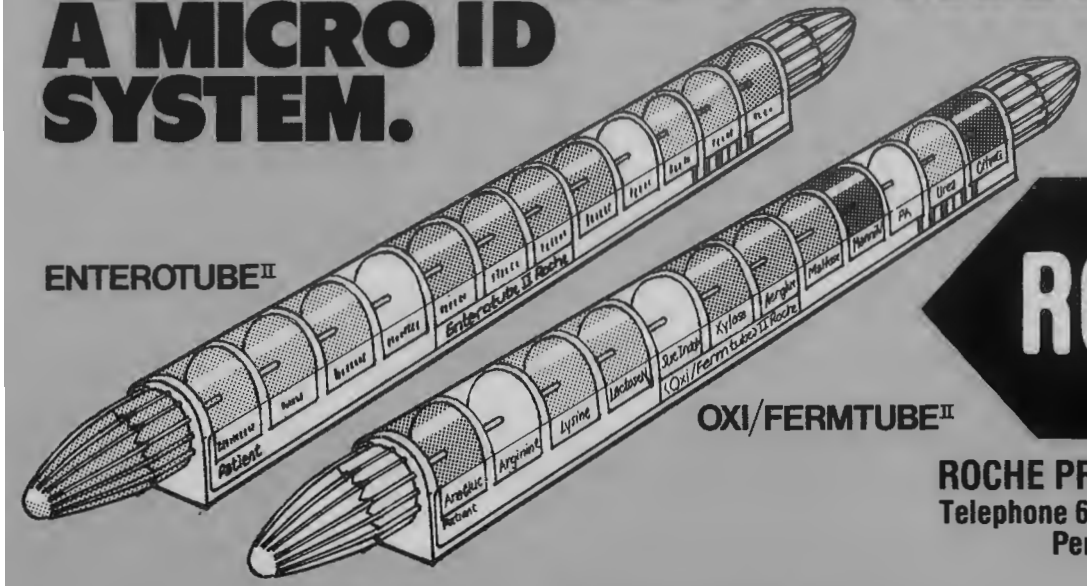

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(b) *Grade Laboratory Officers*

It is a matter of serious concern to the Institute and Charge Technologists that some senior grade positions are not attracting suitable applicants and that application for trainee positions are markedly reduced from previous years. We believe that these factors are directly related to poor salary prospects for Medical Laboratory Technologists. As a result of the Health Service Personnel Commission assuming responsibility for grading in the service and the move to grade positions rather than persons we believe that it is essential to recognise the merit provision for a higher salary than Grade 5 as a substantive Grade 6. Persons currently holding positions which have been awarded the merit step should have their position confirmed as Grade 6. A proposed salary scale which will improve salary prospects and help to retain and attract registered technologists to the profession in outlined below.

10.11.81 + C.O.L.		Proposed Scale + C.O.L.	
Merit	29,758	Grade 6	32,741
5	28,175	5	31,233
	26,888		30,008
4	25,999	4	29,162
	24,909		28,123
3	24,020	3	27,277
	22,931		26,239
2	22,040	2	25,391
	21,150		24,543
1	20,162	1	23,602
	19,271		22,863

(c) *Staff Medical Laboratory Technologists*

The duties and responsibilties of Staff Technologists is inadequately recognised and many are being lost to the hospital service. This has a direct bearing on experienced personnel being available to apply for grade positions. It is proposed that an additional increment be added to the scale in addition to its upgrading in terms of salary which will help to retain staff and allow additional experience to be gained in their particular specialty.

10.11.81 + C.O.L.	Proposed Scale + C.O.L.
	21,859
18,638	21,235
17,982	20,767
17,492	20,165
16,859	19,564

(d) *Sole Charge Allowance*

The Institute seeks an allowance of $750 p.a. for Sole Charge Grade Laboratory Officers. We contend that the duties and extra responsibilities of such persons has not been adequately compensated under the procedure for grading positions. This has not recognised the additional responsibilties of the Grade Laboratory Officer where no Pathologist is available for consultation nor the multidisciplinary nature of the workload. Comparable graded staff in larger laboratories can refer to a Pathologist or their Charge Technologist as required and are generally working in a single discipline. In addition the sole charge person is required to be "on call" for very considerable periods which while partially compensated for by the on call allowance nevertheless adds to stress and places great restrictions on their out of work movements.

(e) *Laboratory Assistants*

The present system of determining entry to the Senior Laboratory Assistants scale is cumbersome and creates anomalies. It had been our contention that the Grading Committee regulations should not have been extended to cover Laboratory Assistants. Now that "positions" are graded it is no longer appropriate for laboratory assistants entry to the senior scale to be based on "personal" grounds.

We contend that a system of entry to the scale based on qualifications is more appropriate and that positioning within both scales should be the prerogative of the employing hospital board.

We have outlined on many occasions the problems associated with laboratory assistants who gain the Q.T.A. qualification but are being paid the minimum adult allowance and have sought to have the situation rectified so that they receive a financial reward for the qualification. Similarly we have argued that a laboratory assistant with the NZCS qualification is entitled to receive the salary increments above the minimum salary for NZCS which is automatic for trainee technolgists and **for all such persons in the State Services.**

Blood Transfusion Services throughout New Zealand are pursuing a policy of employing Registered or Enrolled Nurses. These persons are classified as laboratory assistants under H.S.19. The Senior Laboratory Assistant scale is not adequate to compensate Registered Nurses in those positions where whilst they are not strictly carrying out nursing duties they are making clinical decisions as to donors' suitability to give blood in addition to their phlebotomy duties. In addition they are required to supervise donors connected to blood separation machines for obtaining platelets, granulocyte concentrates, plasmaphoresis, etc.

We propose therefore that the Laboratory Assistant scale shall remain unchanged and that a new Senior Laboratory Assistant scale be introduced with eligibility for entry based on the laboratory qualifications of QTA and NZCS and the nursing qualifications of Enrolled Nurse and Registered Nurse. The justification for extending the scale to $18275 p.a. lies in H.S.21 District Nursing scale to which nurses employed in transfusion services should be entitled. A laboratory worker would take ten years post-QTA qualification to reach this salary. We do not expect that many would reach that position but it improves their prospects. This may have a bearing on whether the proposal by laboratory assistants in the Auckland area to form an industrial union is carried forward.

Laboratory Assistant 10.11.81 + C.O.L.		Proposed Senior Laboratory Assistant + C.O.L.	
(unchanged)			
14,447			
14,026		18,275	
13,546		17,863	
12,989		16,455	
12,145		15,592	
11,026		15,011	
10,217		14,447	NZCS
9,360		13,411	Reg. Nurse
8,503		12,989	
7,646	U.E.	12,145	
6,995	S.C.	11,026	QTA, Enrolled
6,475			Nurse
5,726			
5,318			

Advancement within the scales is by automatic progression or by accelerated progression as determined by the employing board.

(3) **CONDITIONS**

(i) *Hours of Work*

The Institute seeks a reduction in the hours of work for laboratory workers. This is sought to reduce the amount of stress being experienced and to reduce the exposure of workers to infectious diseases notably Hepatitis B and A.I.D.S.

The effects of stress on laboratory workers is well documented overseas and has been discussed in conciliation in previous meetings. New Zealand laboratories are no different to those overseas in this regard. Additional contributing factors in larger hospitals have been the introduction of 16 or 24 hour shifts with their disruptions to life styles, and the demands by intensive care units which result in onerous call back duty where full 24 hour coverage is not possible.

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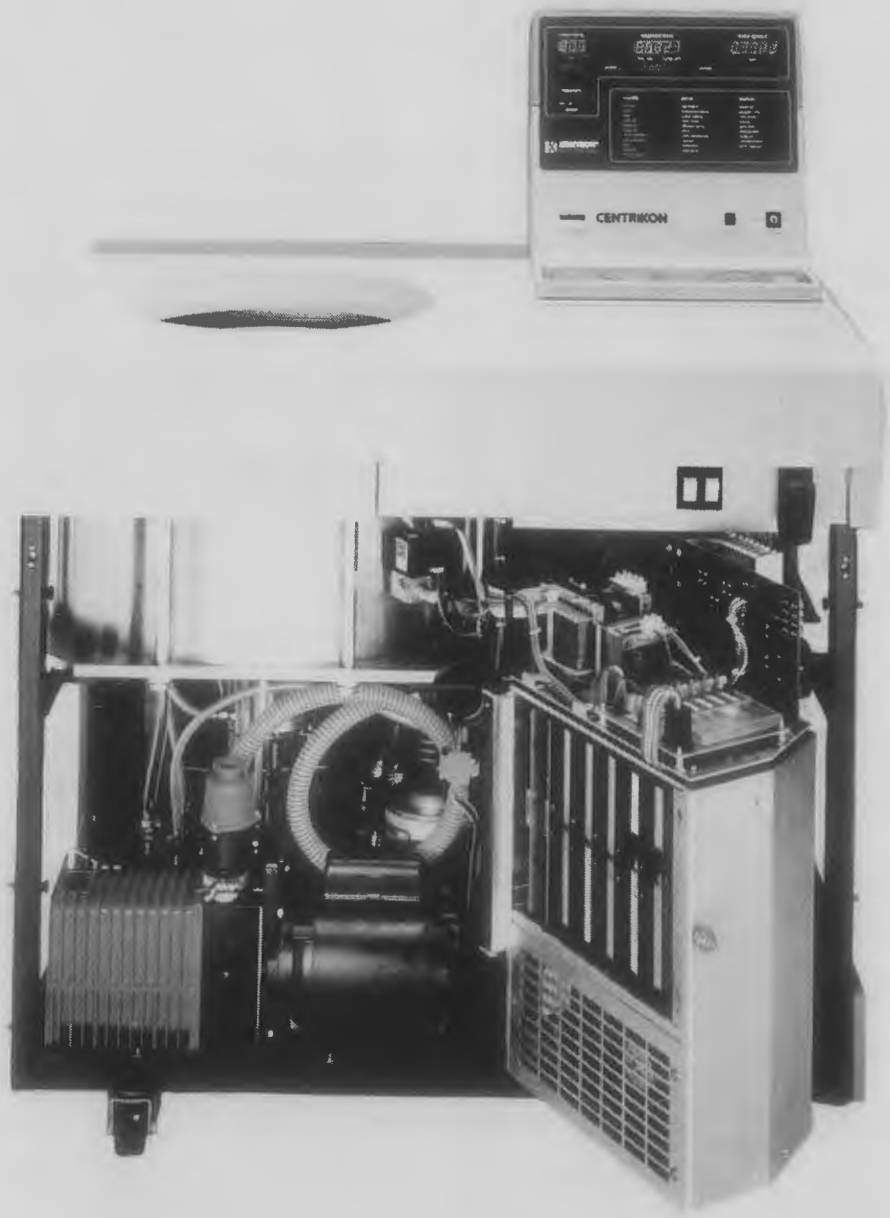

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The dangers of infection to the above diseases cannot be over emphasised. Currently there is little or no protection provided for laboratory staff handling routine blood samples. Experience recently overseas has shown that massive expenditure on such items as Laminar Flow Cabinets will be required to protect staff. We believe that it is necessary to reduce exposure immediately by a reduction in working hours.

Proposed Clause:

Hours of work "Ordinary weekly hours of work shall be 7 hours per day worked on 5 consecutive days. Each daily duty shall be continous except for interruptions for meal periods and rest breaks."

(ii) *Part-Time Employees Call Duties*

There is no provision in H.S.19 for part-time workers undertaking call duties. A provision similar to that contained in H.S.16 (X Ray workers) would overcome any problems in this area.

Proposed wording:

Overtime and Penal Time : Standard H.S.48 except that:

1. Part-time employees (call-backs) —

Overtime rates will only apply where the part-time worker has worked in excess of 7 hours per day or 70 hours per fortnight. Where part-time workers are part of an official on call roster and are called out from their place of residence in emergency circumstances, then they shall be paid on the basis of a minimum of 3 hours at appropriate rates. The length of the call would be measured in respect of actual time worked only, except that outside of the normal hours of duty (i.e. 8.00 am to 5.00 pm Monday to Friday) the length of the call would be measured in respect of actual time worked and reasonable travelling time from the place of residence. The minimum payment prescribed shall apply to each recall, except that:

(1) call-outs commencing and finishing within the minimum period covered by an earlier call-out shall not attract any additional payment;

(2) where a call-out commences before and continues beyond the end of a minimum period for a previous call-out payment shall be made as if the employee had worked continuously from the beginning of the previous call-out to the end of the latter call-out.

(iii) *Transport to work before and after cessation of Public transport*

There is provision for a laboratory worker to be reimbursed for actual and reasonable expenses for transport when called out outside normal hours. The Standard H.S. 48 transport allowance applies at other times. Laboratory workers are required to take taxis home when completing shifts at midnight for personal safety reasons. The transport allowance does not cover the taxi fare. This also applies to workers commencing shifts at midnight and at weekends. These workers are thus penalised for undertaking shift duty which in itself can be onerous in that the majority of the work is urgent. However our chief concern is for the safety of our members. In the Auckland Blood Transfusion Service over 90% of workers have been accosted when leaving work at midnight.

In addition difficulties are being experienced by some Transfusion Service staff in being provided with appropriate accommodation and meals while on mobile blood collections. In order to overcome these problems we suggest that the Transport Allowance provision be amended to include the following clauses:

TRANSPORT ALLOWANCE

Present H.S.19 and H.S.48 except that — Any worker required to commence work after the cessation of public wheeled traffic or before the ordinary time of starting such traffic, and any worker who may work continuously until after the cessation of public wheeled traffic and cease work before the ordinary time of starting such traffic, shall be conveyed to or from his/her home at the expense of the employer or, if he/she uses his/her own transport, he/she shall be paid at the standard mileage rates.

We would point out that these provisions have applied for years to Domestic workers and Hospital Orderlies.

MISCELLANEOUS TRANSPORT PROVISIONS

The following miscellaneous transport provisions shall apply:

(a) A laboratory worker employed in an institution who is required to undertake duty for an indefinite or extended period in another institution or location, shall be transported to that institution or location with his luggage at the board's expense.

(b) Where a board requires a laboratory worker to work temporarily at an institution or location other than where he is normally employed, and this requirement results in additional daily transport expenses for the worker concerned, the board shall reimburse the worker the actual and reasonable additional transport costs incurred.

(c) Where a laboratory worker is required to work temporarily at an institution or location other than where he is normally employed and accommodated, the board shall either provide transport each way each day, or reimburse the worker the actual and reasonable accommodation costs incurred.

(d) Travelling time on the following basis may be paid in the circumstances provided for in subclauses (b) and (c) above, when the time occupied in travelling to the new institution or location exceeds that normally occupied by a laboratory worker in travelling daily to and from his normal place of employment:

  (i) Travelling time falling within the normal hours of duty shall be regarded as time worked.

  (ii) Travelling time falling outside the normal hours of duty shall be paid for at T1 rate, but will not count towards the computation of daily or weekly overtime payments.

(iv) *Uniforms and Protective Clothing*

The present Standard H.S.48 provision is inadequate in present working conditions following the introduction of stringent safety rules in all laboratories as a result of inspections by TELARC. Workers are required to wear suitable footwear and clothing for safety reasons. Many workers in Blood Transfusion Services and venipuncture services are required to wear standard uniforms and footwear. For these reasons a change to the conditions applying to the nursing sector is sought and is as follows:

UNIFORMS AND PROTECTIVE CLOTHING

(1) This clause shall apply to all laboratory workers.

  (a) Where a board requires a laboratory worker to wear a particular uniform, this shall be supplied free of charge but shall remain the property of the Board. Suitable protective clothing shall also be provided at the board's expense where the duty involves a risk of excessive soiling or damage to uniforms or personal clothing.

  (b) The term uniform shall include uniform dresses, trousers, tunics, caps, cape, cardigan or woollen jacket.

  (c) All items of uniform clothing supplied by the board shall be laundered or dry-cleaned at the board's expense, as and when required. Each case is to be determined on its merits by the board concerned.

  (d) (i) Where a board requires a laboratory worker to wear a particular type of shoe, two pairs shall be supplied free of charge to every whole-time worker or an allowance of $79.84 shall be paid in lieu.

  (ii) Six pairs of duty socks, stockings or panty hose shall be supplied free of charge or an allowance of $17.94 p.a. shall be paid in lieu.

  (iii) In the case of a laboratory worker who is employed part-time, a proportionate part of these allowances shall be paid as applicable.

(e) Damage to Personal Clothing — A laboratory worker may, at the board's discretion, be compensated for damage to personal clothing worn on duty, or reimbursed dry-cleaning charges for excessive soiling to personal clothing worn on duty, provided the damage or soiling did not occur as a result of the employee's negligence or failure to wear the protective clothing provided.

(v) *Meal Periods and Rest Breaks*

Laboratory workers particularly in cross matching laboratories are frequently unable to leave for a meal break because they are required to continue with the preparation of blood for transfusion. It is contended that such persons should be compensated by additional payment until a meal break can be taken. There is a precedent for this in H.S.21 Nursing Occupational class.

Suggested wording:

Standard H.S.48 provided that any laboratory worker unable to take a meal break after 5 hours duty shall be paid at time half rate in addition to normal salary from the expiry of 5 hours until the time when a meal break can be taken.

Yours faithfully
B.W. MAIN
Chief Negotiator

The Response

Brian Main, the chief negotiator, reports "The Institute Negotiations Committee met on 18.1.85 and 29.1.85 with a sub-committee of the S.S.C.C. chaired by Mr T. Neilson. As expected most of our claim had been declared "interservice".

(1) H.S.19 is to be revised during the next few months by the Health Services Commission and will be referred to the Institute for comment. It is likely that in the next salary round we will be asked to accept a new hours of work clause to enable rostered shift work to be undertaken.

(2) (a) The Graduate Technologist scale will be brought into line with Grades I and II of H.S.51. This will benefit 4 persons still on this scale.

(b) (c) and (d) No offer was made to these proposals on the grounds that hospital boards had advised the Commission that no recruitment or retention problems were being experienced. Under the 1983 Amendment to the State Services Conditions of Employment Act the only criteria for an increase in salaries is difficulty in recruitment or retention of staff. Our request for a substantive Grade 6 to replace the merit step was rejected on the grounds that this would constitute a salary increase of some 12% and that the Health Service Commission Act allows for the grading of persons as well as positions.

It was claimed that persons in sole charge positions could have their additional responsibilities recognised by the Commission in respect of their position grading. Therefore no allowance was necessary. In order to test this assertion, the Negotiating Committee would recommend that charge technologists where there is no pathologist should apply to the Commission to have their position grade reviewed preferably with their hospital board's support, but if this is not forthcoming on a personal basis.

(e) No offer was received in respect of the Laboratory Assistant scales but a working party will be set up to assess the duties and responsibilities of laboratory assistants in the hospital service vis a vis the DSIR M.A.F. This is to report back by 10.7.85. We have been asked to submit names of senior technolgists one of whom will be chosen to act as a technical advisor.

(3) (i) Our claim for a reduction in work hours was rejected on the grounds that there is no weight of medical evidence to suggest that laboratory workers are more at risk than other hospital staff and that it would constitute a wage increase in excess of the Government guideline.

(ii) Part-time employees (call backs). This claim was accepted with the hours of work being 8 per day and 40 per week.

(iii) Transport Allowance
No offer. The Negotiations Committee is to approach the C.S.U. again to request that this be negotiated with the S.S.C.C.
Miscellaneous Transport Provisions
To be reviewed in conjunction with the working party assessing laboratory assistants.

(iv) No offer except for item (e) which was accepted.

(v) Meal periods and rest breaks
No offer but the working party is to review this when undertaking the laboratory assistant assessment.

In spite of the very spirited efforts of the Committee in negotiation it is disappointing that so little has been achieved. It does illustrate however how difficult it is to make any progress when the claim is inter-service. There is some hope for progress in the laboratory assistant area as it was acknowledged that there are problems particularly in the Blood Transfusion Service and Mortuary areas. We are able to resume negotiations on or after 10.7.85 and the Annual General Adjustment will apply to all laboratory workers when finalised."

The Negotiations Committee recommends that:

1) Laboratory Assistants should apply for entry to the Senior Laboratory Assistant scale once they have met the criteria of being on the top of the Laboratory Assistant scale for 12 months and hold the Q.T.A.

2) all sole charge Graded Laboratory Technologists should seek a review of their grading if they consider their grading does not recognise their extra clinical responsibilities in the absence of a Pathologist.

3) any laboratory worker who requires clarification of their position should contact any member of the Negotiations Committee.

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MEMBERSHIP SUB-COMMITTEE REPORT NOVEMBER 1984

Membership

Since our August meeting there have been the following changes:

		Aug 84	Nov 83
Membership as at 09/08/84	1406	1378	1389
LESS Resignations 17	18	27	6
Death 1			
		1351	1383
PLUS Membership Applications 18	23	55	39
Lost Membership — re-enrolled 5			
TOTAL MEMBERSHIP as at 19/11/84	1411	1406	1422

Applications for Membership as at 14 November

Miss T. Crosby, Tauranga; Miss P.D. Finch, Palmerston North; Miss K.M. Fitzgerald, Tauranga; Miss C. Henderson, Auckland; Ms H. Hobson, Nelson; Mrs M.J. Howe, Australia; Miss S.C. Keller, Christchurch; Miss M.R. Le Levre, Auckland; Miss K.H. Mavor, Oamaru; Mr C.J. McKenzie, Auckland; Miss W.M. Pearce, Hamilton; Miss J.A. Scott, Tauranga; Miss L.A. Scott, Palmerston North; Mrs J.A. Sisson, Christchurch.

Applications for Associateship

Miss M. Nahna, Te Kuiti; Mrs J.M. Nicholson, Wellington; Miss L.A. Tate, Auckland; Miss S.P. Robertson, Christchurch.

Resignations

Mrs S. Darby, Palmerston North; Mr T.K. Offord, Wellington; Mr A.N. Green, New Plymouth; Miss A.T. Batkin, Auckland; Miss M. Martyn, Australia; Mrs T.M. Smith, Wellington; Miss L. Pearce, Tauranga; Mrs M. Scutts, Thames.

Gone no Address or no Resignation Received

Miss A.Y. Holmes, Mrs B.R. Little, Miss L.M. May, Miss S. Fage, Mr C.E. Young, Mr T. Harris, Miss A.M. Ridley.

Deceased

Mrs A.A. North, Auckland.

CORRESPONDENCE

Dear Sir,

I am in receipt of your letter of the 11th October informing me of Council's decision to grant me Fellowship Status.

I would like to thank the Fellowship Committee and Council for this honour.

Yours sincerely,
Alan Harper

Dear Sir,

Thank you for your letter dated 11th October in which you inform me of the N.Z.I.M.L.T. Council's decision to grant me Fellowship of the Institute.

I would be grateful if you would convey my appreciation and thanks to the Council for their action.

Yours faithfully
Gilbert Rose

Dear Sir,

I found your letter dated 4th October 1984 a very pleasant surprise and I would like to thank the Institute for the award. Incentives for technologists to become more professionally involved are few and far between and I would like to commend the Institute on this policy.

With the introduction of new technologies in the histopathology area, there has appeared a wealth of ideas for developmental work. There are immunoperoxidase kits commercially available that require evaluation, new histochemical techniques to try, plastic section technology etc. I have tried to urge fellow technologists, using these technologies, to critically evaluate them, develop them and then "put pen to paper" and document their findings in their own Journals, so that we all can appraise and learn from their findings. Too often we hear "Oh, we had that same problem last year and this is what we did...". Why aren't these comments developed and published?

I believe that the New Zealand Institute offers a valuable incentive to technologists to become more professionally involved and I would like to again thank you.

Yours sincerely,
Anthony F. Henwood
Principle Science Officer
Histopathology Department
Dept of Veterans' Affairs
P.O. Box 1652
Adelaide 5001

Health Service Personnel Commission

HSPC Circular 1984/35

Chief Executives of Hospital Boards

Dear Sir/Madam

Classification and Grading

Background

The Health Service Personnel Act 1983 provides for the Health Service Personnel Commission to be responsible for establishing, and conducting a regular review of, the classification and grading of positions or where appropriate, persons in the Health Service. The relevant sections of the legislation covering classification and grading are sections 26 to 29 and section 40. They do not apply to Medical Officers or employees covered by awards or agreements under the Industrial Relations Act 1973.

Section 26 sets out the responsibilties of the Commission in conducting a review. These are to:

(a) ascertain whether or not there is a need to adjust the scope and content of occupational classes, or the salary scale or grading pattern, or conditions of employment, of any occupational class;

(b) ensure that all positions are placed in the occupational class most closely related to the nature of the duties involved;

(c) for every position in each occupational class determine a grade appropriate to the nature and relative importance of the duties and responsibilities of, and the level of skill required for, the position

and ensure every person appointed to a position in the Health Service is placed in an appropriate occupational class and graded according to the level of skill of responsibility required to be exercised.

To assist in determining the appropriate gradings, the Commission may establish advisory/technical committees. The Commission has decided that these committees will be ad hoc and will not include members nominated by service organisations.

Applications for Regradings

A review of grading may be sought by either a board in its capacity as employer or by an employee. The distinction between the two is important in that while an employing board may lobby the commission to whatever extent it wishes, it has no recourse to any other body. The employee on the other hand has available under Section 27 of the legislation the right to apply to the Commission for a review of grading of the position he occupies or his personal

grading if the position is not graded, if he has not applied to a Grading Review Committee constituted under Section 28 within the preceding five years and there is scope for further advancement within his occupational class.

On receipt of the application, the Commission is required to review the case and notify the employee in writing of the result of the **review.**

Grading Review Committees

If an employee is not satisfied with the Commission's decision, he may within 30 days request that his application be referred to a Grading Review Committee established by the Commission. The Grading Review Committee is to consist of an independent chairman and two members appointed by the Commission, one after consultation with the Hospital Boards' Association and the other on the nomination of the service organisation representing the interests of the employee whose application is being considered.

Once the Grading Review Committee has completed its review of a grading, it may recommend to the Commission that the employer:

— decline to alter the present grading; or
— appoint the applicant to the position at the new grading; or
— declare the position vacant and fill it in any manner authorised under the Act; or
— promote the employee to the new grade, where the position is not graded.

If the recommendation of the grading review committee is accepted by the Commission, the Commission is required to advise the employer of its decision, and the employer shall comply with that decision. The commission is also required to send a copy of the grading review committee's recommendations to both the employer and the employee.

The Commission is not required to accept the recommendation but where the Commission's decision differs from the recommendation the reasons for its decision must be sent to the employee and the employing board.

Health Service Appeal Board

Where the Commission's decision differs from the recommendation of the Grading Review Committee the employee may apply to the Health Service Appeal Board established under Section 30 of the Act. Any decision made by the Appeal Board is binding on the Commission, the employing board and the employee.

Reviews of Grading

The legislation requires that reviews of gradings be conducted on a regular basis. The commission has decided that this shall be by a continuous review rather than the once yearly system that applied in the past. Boards are therefore expected to regularly review the gradings of positions and employees where positions are not graded and there is provision in the determination for promotion on the basis of merit or some other criteria, and submit applications for regradings as the need arises. Reviews of gradings should only be requested when there is a substantial change in the level of responsibility vis a vis others in the same occupational group or where, in the case of personal grading there is clear evidence that the criteria set down in the determination has been met.

In the case of Scientific Officers and Technicians, biennal reviews of gradings will still be carried out and a separate circular will set out the procedure to be followed for these employees.

Submissions for Reviews

Where positions are graded submissions must be based on the duties and responsibilities of the position. Submissions based on the personal qualities and attributes of the employee will not be considered.

In cases where positions are not graded, and promotion is based on evidence of merit or some other criterion in the determination, the submission must be on the basis of the determination requirements eg if working in a specialised area of work is a requirement then this must be shown.

Hospital boards are expected to submit a well documented and argued case to alter an existing grading.

Where positions are graded, the submission should cover the following areas:

1. **Job Content** — an outline of the complexity and diversity of the position including the types of decisions the employee may be required to make, the degree of accountability the employee has for those decisions and the consequences of making an error. It should be made clear to whom the employee is directly responsible and what place that position has in the board's organisation. An up-to-date job description should be attached to the submission.
2. **Staff Management** — the number of staff the employee is responsible for and their gradings and occupational groups. The authority the employee holds for staff selection, appointments and discipline including dismissal. The extent of the supervisory responsibility the employee exercises over staff (direct or indirect). Involvement in training, rostering and industrial relations.
3. **Financial Control** — the delegations held for purchasing goods and services. The degree of control the employee has over his or her own budget, and the amount of that budget. The degree of participation by the employee in compiling the budget.
4. **Participation in Policy Making and Planning** — an outline of the planning the job requires in regard to the objectives, policies, programmes and procedures of the board and the influence the employee has in establishing policies etc. Also the extent to which the employee is required to interpret Board policies.
5. **Communications** — a broad outline of the level of contacts required of the employee. The types of people communicated with both within the board and outside.
6. **Qualifications** — the qualifications and experience required for the position.
7. **Representational Role** — the extent to which the position involves representing the board — what forums — role in such forums (member of committee, chairperson) — ability to commit board.

If possible a comparison should also be made between the position and other positions within the board in the same occupational group having regard to the factors outlined. As the Commission in assessing gradings may only consider positions within the same occupational class comparisons made with other positions in the board's service are invalid and should not be put forward as justification for a review.

Where positions are not graded the submission should cover the requirements of the determination. Where merit is a criterion for promotion and it is undefined, the submission should demonstrate that:

— The employee has been on the maximum of the grade for at least 12 months.
— The employee is performing at a level higher than that normally expected of employees in that grade.

Because of the range of occupational groups involved, it is not possible to list every item that should be covered in every case. It may well be necessary for some positions to add information in order to cover all aspects of the job.

It is appreciated that the procedures set out in this circular represent a significant change over past practice. It will obviously take time for boards and the Commission to become familiar with the new system. The Commission will be very willing to assist and advise with any queries or difficulties which may be encountered by boards. Boards should not hesitate to seek clarification from the Commission on any points of doubt.

Yours faithfully
H.F. Smith
Chief Executive

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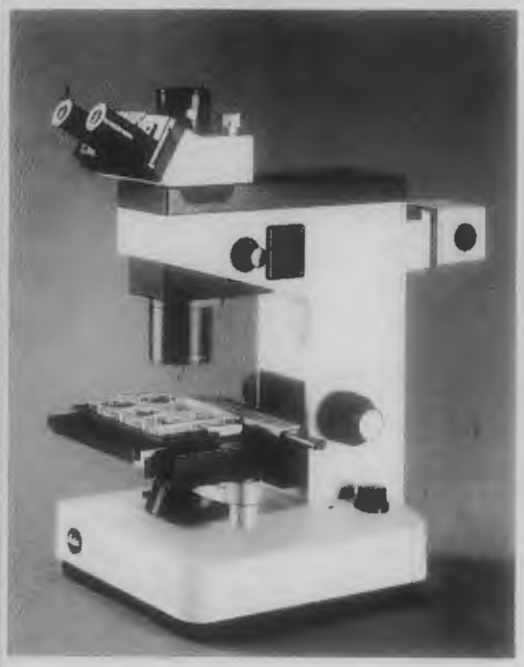

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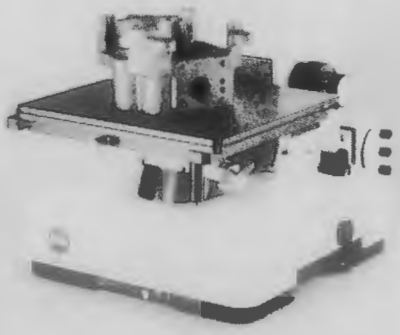

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Thank You

The membership files of the NZIMLT will be transferred from the computer at Hamilton Medical Laboratory to a professional computer bureau, Online Business Management Ltd in Auckland as from April 1st, 1985.

The Institute wishes to express its gratitude for the excellent service that the Hamilton Medical Laboratory has provided for us over many years. Many thanks for a job well done.

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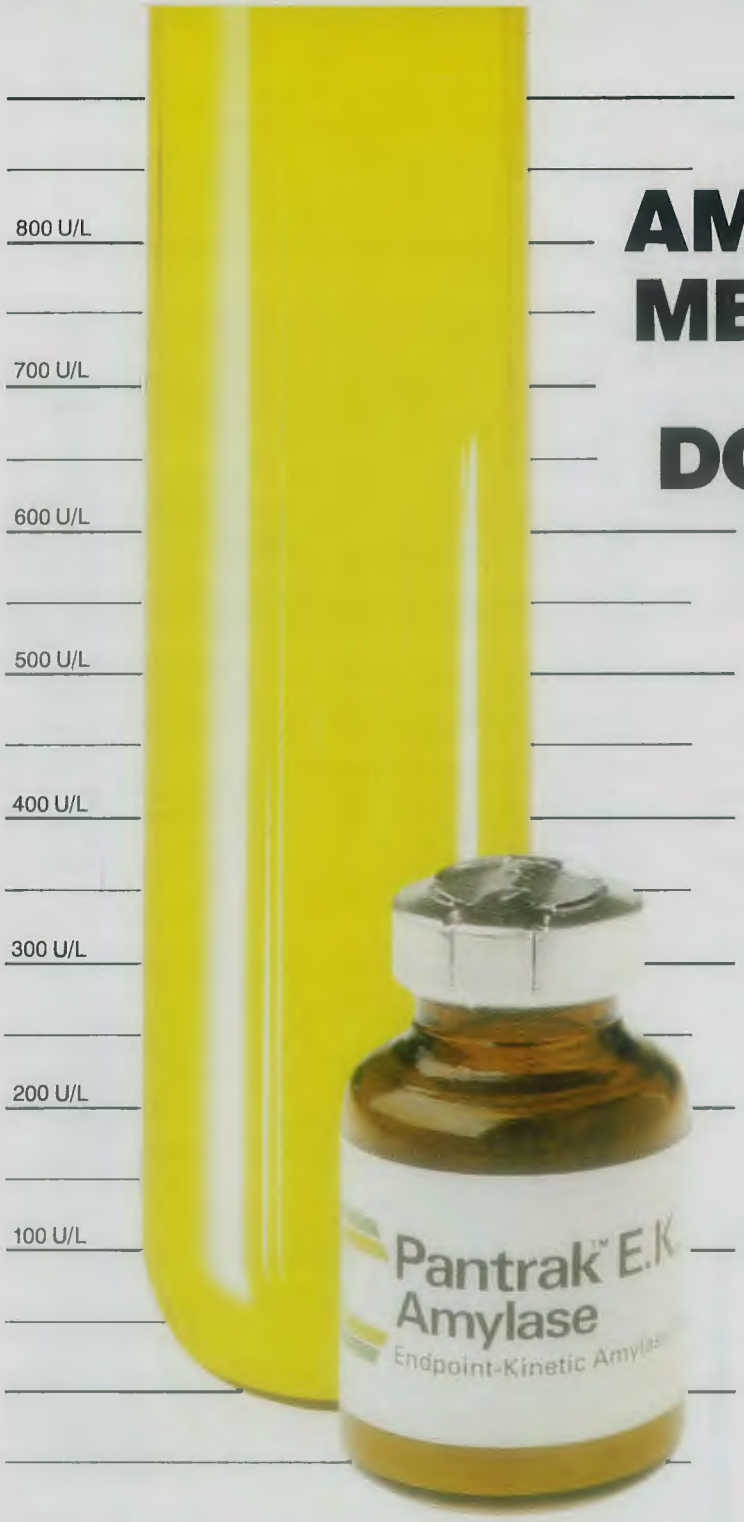

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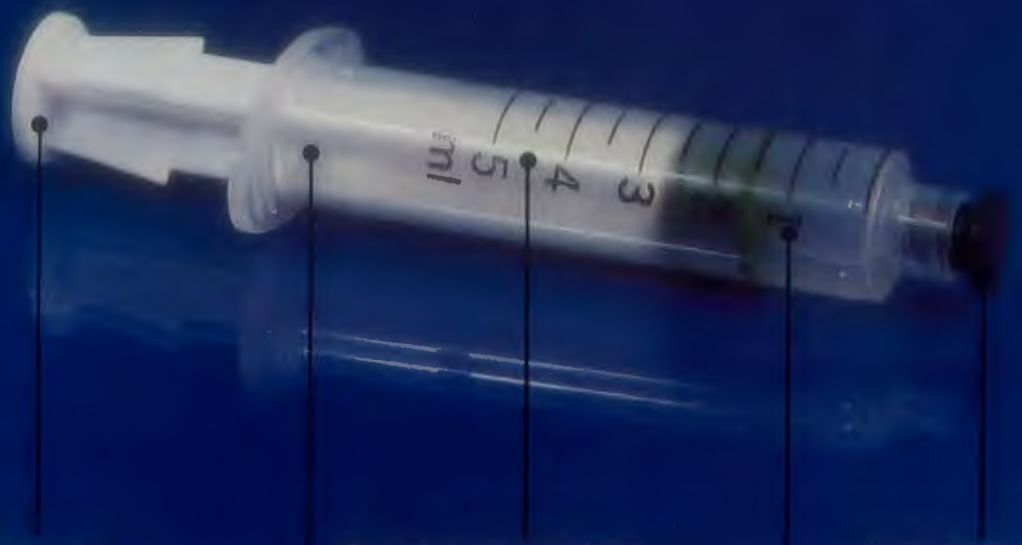

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