DAR

THE NEW ZEALAND JOURNAL OF MEDICAL LABORATORY TECHNOLOGY

THE OFFICIAL PUBLICATION OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY INCORPORATED.

EDITOR:
John Case

SUB-EDITORS:
J. Rees H. C. W. Shott

ADVERTISING MANAGER:
F. C. Kershaw

CIRCULATION MANAGER:
E. K. Fletcher

JOURNAL REPRESENTATIVES:
Auckland — J. Holland
Christchurch — A. C. Titheridge
Hamilton — M. G. Harper
Wellington — W. Aldridge

VOLUME 18. No. 3 OCTOBER, 1964

NEW B.D.H. CLINICAL REAGENTS

B.D.H. has pleasure in announcing a new range of clinical reagents for both automatic and other methods of analysis containing groups of solutions for six different determinations:

ALKALINE PHOSPHATASE in serum
CARBON DIOXIDE in serum or plasma
GLUCOSE in biological fluids
TOTAL PROTEIN in serum or plasma
UREA NITROGEN in biological fluids
URIC ACID in serum or plasma

The use of these solutions enables tests to be carried out with accuracy, speed and economy.

ACCURACY The solutions are prepared in large quantities and accurately standardised.

SPEED No weighing or dissolving of reagents is required; working solutions are prepared using only pipettes and volumetric flasks.

ECONOMY Most of the solutions are in concentrated form, each litre supplied yielding on dilution 10 litres of working solution, with consequent savings both in cost and in storage space.

A free booklet, describing the solutions in detail and listing other B.D.H. reagents for clinical use, will gladly be sent on request.

THE BRITISH DRUG HOUSES LTD.
B.D.H. LABORATORY CHEMICALS DIVISION · POOLE · ENGLAND

CR4

Diagen

TWO-SEVEN-TEN

Reagent

A PRODUCT OF BRITISH RESEARCH AND MANUFACTURE

The use of TWO-SEVEN-TEN reagent simplifies the technique of routine testing during anticoagulant therapy, since it is only necessary to add a measured volume of the patient's blood or plasma to a measured volume of the combined reagent and observe the clotting time. The clotting time is then converted into percentage activity by reference to the calibration curve supplied with each batch of reagent. This obviates the necessity of preparing brain thromboplastin or purchasing commercial thromboplastins with the attendant difficulties of standardising, re-checking, deterioration, and contamination with factor VII or inhibitors. There is also considerable saving in technical labour, which is of special importance to the small laboratory where it may be difficult to devote sufficient time to careful standardisation for a small turnover of patients on anticoagulant therapy.

Sole New Zealand Agents:—
ETHICALS LTD.,
P.O. BOX 18-040, GLEN INNES.

Made in England by:—
DIAGNOSTIC REAGENTS LTD., THAME, OXON.

URASTRAT

(WARNER-CHILCOTT)

urea nitrogen assay system...

uncomplicated accuracy

The Urastrat® chromatography strip below contains, in precise amounts, all the reagents required for one fully quantitative Urastrat urea nitrogen assay.

Serum volume: 0.2 ml.

Time required: 1 minute working time, 30 minutes incubation at room temperature.

40-test boxes	**40/-**
250-test boxes	**225/-**

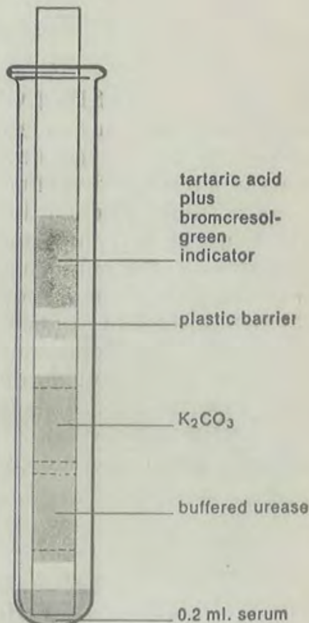
Outwardly simple, the Urastrat assay is actually a precisely controlled sequence of chemical reactions closely paralleling those of the Conway microdiffusion method.

As the serum rises up the Urastrat strip by capillary action, a zone of buffered, high-potency urease (*specially purified by dialysis*) splits the urea present, yielding ammonia in quantity proportional to the urea nitrogen concentration.

Next, K_2CO_3 releases the ammonia as a free gas. Ascent of the serum stops at the plastic barrier, but the gaseous ammonia migrates upward to be trapped by the tartaric acid in the indicator band, causing a pH change which turns the bromcresol-green indicator from yellow to blue.

The more urea nitrogen originally present, the more ammonia is trapped and the higher the blue frontier rises on the indicator band.

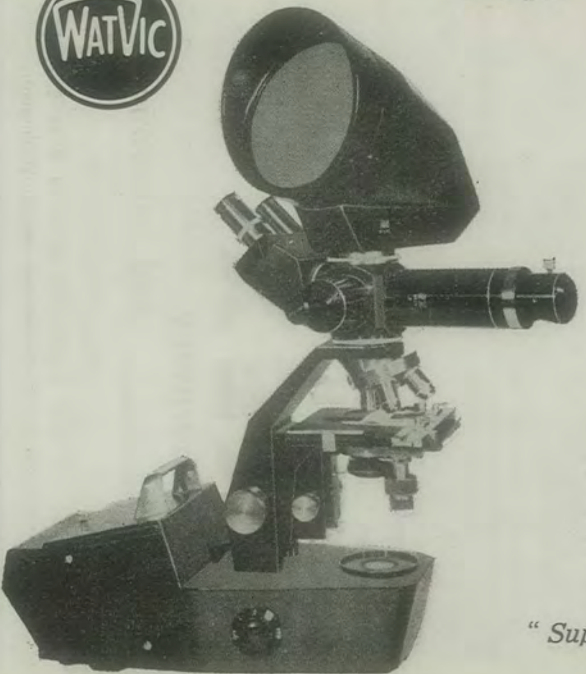
After 30 minutes incubation at room temperature you measure the height of the color change in millimeters, translate into mg. urea nitrogen/100 ml. serum by a simple calculation.



For full information write to

William R. WARNER and Co Ltd
P.O. BOX 430 AUCKLAND

GILLETT & SIBERT CONFERENCE MICROSCOPE



FEATURING . . .

- High Intensity, Quartz Iodine light source.
- Viewing Screen, 6in in diameter.
- Full range of Optics for Visual and Photographic Work.
- Projection Head, usable with all objectives.

Full details and information on this new range of Microscopes available from . . .

WATSON VICTOR LTD

16 THE TERRACE — WELLINGTON

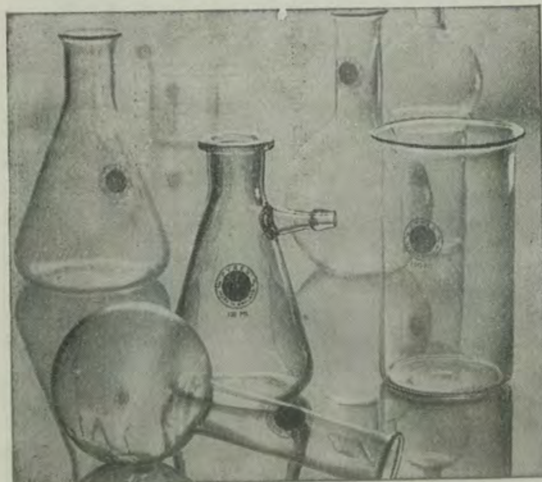
ALSO AT AUCKLAND, CHRISTCHURCH AND DUNEDIN.

"Supplying Equipment and Technical Service since 1888."

A WELL-AIMED BLOW BY PYREX

A machine called the Turret Chain made these: it automatically makes blownware such as flasks, beakers and other types of laboratory ware, to a very high standard of uniformity. It's the only machine of its kind in Europe. Just an example of how PYREX, the first and most important source of borosilicate glassware in this country, use the latest and best processes available. PYREX are always improving their production methods to attain even higher standards of quality. This is one good reason (among many) why everyone who is looking for quality glassware, looks for PYREX.

Pyrex beakers and flasks



are made in all practical sizes and shapes for students, routine or research work.

Extremely low expansion coefficient virtually eliminates breakage from thermal shock allows more robust construction giving greatly increased mechanical strength

High stability against attack from water and all acids (except hydrofluoric and glacial phosphoric)

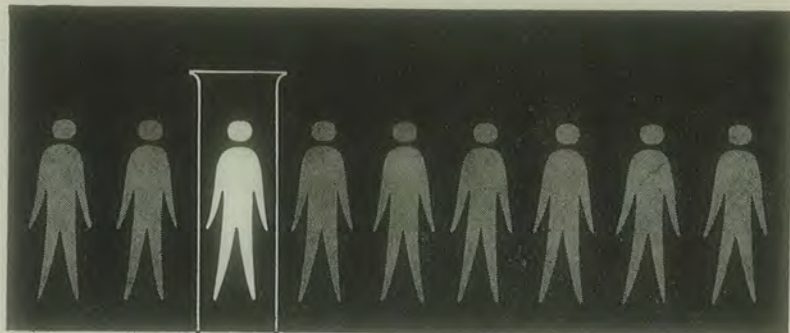
Therefore durable, accurate, economical, dependable, most used.

ENGLISH PYREX

Regd. Trade Mark

Laboratory and scientific glass

N.Z. Agents: Messrs. F. O. & H. S. HART, 9 Holland Street, Wellington.



to detect bleeders before surgery

a new tool for new dependability

Platelin™
standardized platelet factor reagent

Newer tests have been devised to replace the Lee-White Clotting Time, which is known to miss nearly 50% of proven hemophilia cases.(1). These tests are far more dependable indicators of potential bleeders than the routine bleeding and clotting times. They are relatively rapid and simple. But they require a rigidly standardized platelet factor reagent. Until now, no such reagent has been readily available.

PLATELIN is platelet factor reagent, rigidly standardized against normal plasma, against DIAGNOSTIC PLASMA Warner-Chilcott and against plasmas deficient in stage one clotting factors.

Using PLATELIN, any worker skilled in the technique of the Quick One-stage Prothrombin Time can rapidly perform either of two new and sensitive coagulation screening tests:

The Hicks-Pitney Test(2)—a rapid, simplified screening version of the Thromboplastin Generation Test, especially adapted for routine use, which duplicates the extreme sensitivity of the TGT. The only commercial reagents needed are PLATELIN and DIAGNOSTIC PLASMA Warner-Chilcott.*

The Partial Thromboplastin Time Test(3) — similar in procedure to the One-stage Prothrombin Time, and approximately equal to the Prothrombin Consumption Test in detecting bleeders. Only one commercial reagent is required: PLATELIN.

Complete directions for performing and interpreting both the Hicks-Pitney and the Partial Thromboplastin Time tests are included with each package of PLATELIN.

Take advantage of this new advance in coagulation research. Order a supply of PLATELIN today.

Each vial of PLATELIN is sufficient for 12 Hicks-Pitney or 25 Partial Thromboplastin Time tests.

Boxes of 10 vials, 2.5 ml. size, 60/-.

References: 1. Wilkinson, J. F.; Nour-Eldin, F.; Israels, M. C. G., and Barrett, K. E.: **Lancet** 2:947 (Oct. 28) 1961.
2. Hicks, N. D., and Pitney, W. R.: **Brit. J. Haem.** 3:277, 1957.
3. Langdell, R. D.; Wagner, R. H., and Brinkhouse, K. M.: **J. Lab. & Clin. Med.** 41:637, 1953.

* In addition to its use as a reagent in the Hicks-Pitney test, DIAGNOSTIC PLASMA Warner-Chilcott remains the **normal plasma of choice for quality control** of the one-stage prothrombin time and other coagulation tests. Make sure your supply of DIAGNOSTIC PLASMA Warner-Chilcott is adequate.

William R. WARNER and Co. Ltd.
P.O. Box 430, Auckland

DADE REAGENTS, INC. **MIAMI, FLORIDA, U.S.A.**

Manufacturers of high quality biological and laboratory products.

BLOOD SERUMS

A complete range of Blood Grouping and Rh typing serums as well as other Blood Typing serums and Immunohaematological Products is available.

CLINICAL CHEMISTRY CONTROLS

DADE Control Serums for controlling the more common clinical chemistry procedures:—

LAB-TROL	"The Known Specimen" in the normal range for Blood Chemistry Control.
PATHO-TROL	"The Known Specimen" in the abnormal range for Blood Chemistry Control.
ENZA-TROL	The Known Enzyme Test Control.
CHOLES-TROL	A stable liquid control for Cholesterol procedures.
IODO-TROL	The Standardised Control for Protein-bound Iodine procedures.

DIAGNOSTIC REAGENTS

Include Lactic Dehydrogenase, Phosphatase and Serum Transminase Reagents.

COAGULATION PRODUCTS

Specially prepared for use in Coagulation Studies — Standardised Normal Plasma, Cephaloplastin and Activated Thromboplastin.

CALIBRATED LABORATORY GLASSWARE

White Cell, Red Cell, Micro and Rh Titer Pipettes, Accupettes and Thrombin Tubes.

Available ex stock from the Sole N.Z. Agents:

GEO. W. WILTON & CO. LTD.

P.O. Box 367,
WELLINGTON.

P.O. Box 1980,
AUCKLAND.

Further Information and Literature Available on Request.

OXOID ARE FIRST AGAIN

Tablets are one of the unique features of the Oxoid range. No other form is as convenient for preparing quantities of media in small units such as test tubes. Each tablet contains an exact preweighed quantity of material sufficient to make up 10 mls. of tubed medium. For preparing larger quantities without weighing, Oxoid also package many of their media in hermetically sealed, tropically tested sachets, pre-weighed to make up 500 mls. These are particularly useful in warm and humid climates where, in addition to being convenient, they eliminate waste due to caking and spoilage.

MANUFACTURED BY THE OXOID DIVISION OF OXO LTD., LONDON

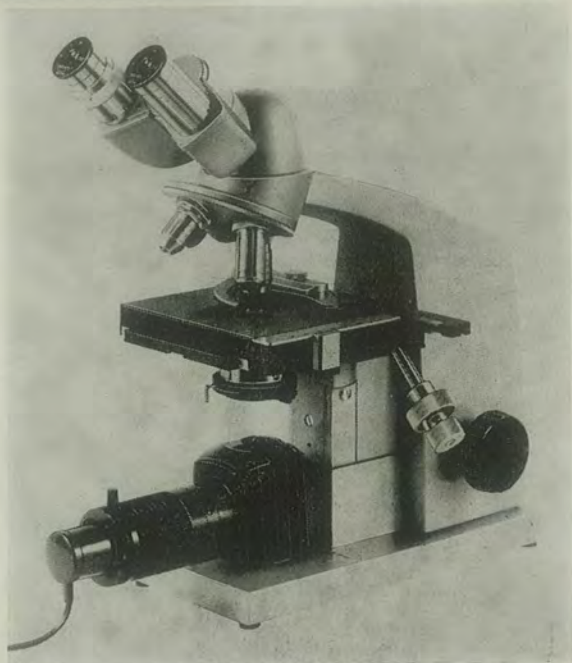
Sole New Zealand Agent:

Edwin A. Piper Ltd., 4 Rata Road, Cheltenham, Auckland, N.1
Telephone: 70-040. Telegraphic address: "EAPAGENT" Auckland

OXOID **CULTURE MEDIA**
for progressive laboratories

REICHERT
WIEN

PRESENTS
the NEOPAN microscope



MODERN STYLING offers rigidity, clean lines, maximum working comfort.

MODERN DESIGN places minimum controls readily into the operator's hands. The ultra advanced movements of the SIMULTAN drive are completely different from any other system: COURSE FOCUS—turn control knob.
FINE FOCUS—slide control knob back or forward.

Separate independent movements positively prevent accidental incorrect focussing

ROBUST CONSTRUCTION, using ball bearings, special lubricants, metals, etc., make the NEOPAN practically maintenance-free.

Ready interchangability of the tubus, condensers, etc. permit the NEOPAN to handle any type of examination. The NEOPAN is ideal for all types of fluorescence examinations, for projection, phase, darkfield, etc., etc.

Combinations to suit you will be quoted on request:

YOU will be amazed at the competitive price of the NEOPAN.

SEE IT!—the new shape will appeal to you
TEST IT!—the ease of operation, the exceptional accuracy will impress you
COMPARE IT!—the high quality, the price will convince you that the NEOPAN will be YOUR new microscope!

REICHERT QUALITY WITHOUT COMPROMISE . .

REICHERT SERVICE CENTRE OF NEW ZEALAND

Box 171 Dunedin and Box 1498 Christchurch.
N.Z. Sole Distributors for *FRIGISTOR* stage & knife coolers.

Q-ESS

PRE-WEIGHED, FOIL-PACKAGED, DEHYDRATED CULTURE MEDIA

PRE-WEIGHED QUANTITIES OF POWDER, SEALED UNTIL THE MOMENT OF USE, IN LIGHT AND MOISTURE - RESISTANT FOIL ENVELOPES EACH CONTAINING A QUANTITY SUFFICIENT TO MAKE A SPECIFIC VOLUME OF FINISHED MEDIUM.

Features:

1. **EXACT WEIGHTS FOR CONVENTIONAL VOLUMES . . .**
   Eliminates Accidental Errors.
2. **SEALED TILL THE MOMENT OF USE . . .**
   Just Snip or Tear Open, and Use.
3. **EFFECTIVE LIGHT BARRIER . . .**
   No Degradation of Nutritional Components.
4. **EFFECTIVE MOISTURE BARRIER . . .**
   No Changes in Bulk Density Due to Ambient Humidities.
   No Caking — Powder at Optimum Conditions for All Requirements.
   Nutritional Value Maintained.
5. **ELIMINATES WEIGHING, MANY STEPS AND USE OF EQUIPMENT OR ACCESSORIES.**
6. **A PRECISE, RAPID, TIMESAVING PROCEDURE . . .**
   Instructions on Each Envelope.
7. **AN INVENTORY SAVER ON MEDIA INFREQUENTLY USED.**
8. **A PREREQUISITE FOR MEDIA USED DAILY . . .**
   Significantly Multiplies Savings of Time and Benefits Secured From Reducing Spoilage and Waste. Provides Media of Uniform Characteristics and Maximum Performance.

Sole Agents for Baltimore Biological Laboratories:

BIOLOGICAL LABORATORIES LTD.

Woodside Avenue, Northcote.
Private Bag, Northcote, Auckland.
Telephones 18-125 and 19-748.

NOW AVAILABLE
IN NEW ZEALAND!

PREGNOSTICON

2-3 hour

Pregnancy Diagnostic Reagent

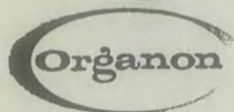
Based on immunological principles, the Pregnosticon Test is extremely reliable and can be used in the early stages of pregnancy. The result is apparent in 2-3 hours.

PREGNOSTICON IS:—

* Equally as accurate as the biological tests.
* Rapid and specific.
* Far simpler to perform.
* Inexpensive.

PREGNOSTICON TEST

Detects Human Chorionic Gonadotrophin (HCG) immunologically in the urine of pregnant women. It requires no experimental animals and no complicated laboratory equipment. Pregnancy can be detected as early as eight days after the first missed period.



Organon Laboratories Ltd., England.
Literature and full details available on request to the New Zealand Distributors:

DOMINION DENTAL SUPPLIES LTD.,

Auckland, Wellington, Christchurch, Dunedin.

THE NEW ZEALAND JOURNAL OF MEDICAL LABORATORY TECHNOLOGY

VOLUME 18. No. 3. OCTOBER, 1964.

The New Zealand Institute of Medical Laboratory Technology (Incorporated).

Office Bearers 1964-65

PRESIDENT
H. G. Bloore
Clinical Laboratory,
Wairau Hospital,
Blenheim.

VICE-PRESIDENTS
M. McL. Donnell
Miss J. Mattingley

SECRETARY
J. D. R. Morgan
Pathology Department,
Wakari Hospital, Dunedin.

TREASURER
D. J. Philip
Pathology Department,
Middlemore Hospital,
Auckland.

COUNCIL
C. W. Cameron
E. K. Fletcher
H. E. Hutchings
R. T. Kennedy

The Journal is published thrice yearly (in April, July and October) and is distributed without charge to all financial members of the N.Z.I.M.L.T. (Inc.). Subscription to the Journal for non-members is ten shillings per year or four shillings per single issue postage paid.

Communications primarily concerning the Institute should be addressed to the Secretary.

Intending contributors to the Journal should address their communications to the Editor at the Department of Pathology, Medical School, Dunedin. Copy must be in the hands of the Journal Committee by not later than the first of the month preceding the month of publication.

Enquiries regarding advertising rates should be addressed to the Advertising Manager at the Microbiology Department, Medical School, Dunedin.

Contributions to the Journal do not necessarily reflect the views of the Editor, nor the policy of the Council of the Institute.

Training and Examinations

Once more a Conference has come and gone. The topics of training and examination have been discussed, dissatisfactions expressed, and few concrete improvements have been suggested. This may be because efforts, so far, have been mainly directed towards endeavouring to bend existing arrangements to fit changing conditions. It is time to look objectively at these problems and to clarify our thinking as to what we, as an Institute of Medical Laboratory Technology, want; and how to seek satisfactory solutions.

The Institute has not fulfilled its proper role in this field. It has been led rather than having provided a lead. There has been an increasing tendency to regard the provision of adequate lectures and correspondence courses as the means of raising standards of training. These have their place, but the regrettable feature of the trend is the emphasis on theory, resulting in a neglect to comprehend the fact that medical laboratory technology is essentially practical work—it is *technology*. An adequate theoretical background to the work is essential, but theory is not an end in itself, it is only the means to an end. However, the major error of thought about training is the view that it is possible under today's conditions to train a technologist to a satisfactory standard in all aspects of laboratory work. The scope of medical laboratory technology has so changed, and the standard of technical competence required has so increased, that it is no longer possible to think along these lines. This fact has been recognised to varying degrees by our counterparts in many countries overseas, but we have stubbornly held to our conviction that, for some reason, the needs of New Zealand hospitals are different.

It is becoming ever more apparent that, after a period of initial grounding in the principles of medical laboratory technology, training should be concentrated on one aspect of the work for the purpose of examination at Associate level or higher. Only by this means can technologists be trained to a satisfactory standard as demanded by present-day medical laboratories.

The principle of specialisation being accepted as necessary, the question of examinations must be studied. What is an examination for? It is to determine the knowledge of the candidate, to a given standard in a given subject. The subject and standard are defined by a syllabus. The major weakness of the present system of examinations is that it is not possible to examine anyone thoroughly enough in all aspects of laboratory work, on only two occasions in five years. It is just as impossible to *train* a person adequately in all branches of the work in five years. A sensible suggestion, surely, is that papers in all subjects should be set each year at all levels. Candidates could then be

thoroughly examined in their work at reasonable intervals, by sitting the papers appropriate to their course and stage of training. This represents a revolutionary alteration to present arrangements, but it has much to commend it both from the academic and administrative viewpoint.

Specialisation in training must come if standards are to be maintained. Satisfactory examining can only be achieved by examinations at more frequent intervals and, we can be sure, we shall not command the respect we think we deserve from our pathologists until we see to it that technologists emerge from their qualifying examination with a higher standard of knowledge and competence than is the case at present.

M. McL. DONNELL

Vacancies

COOK HOSPITAL BOARD
GISBORNE

FULL-TIME PATHOLOGIST

APPLICATIONS are invited from Qualified Medical Practitioners for the position of full-time Pathologist at Cook Hospital Gisborne.

The salary payable will be according to grading, which will be determined by the Grading Committee appointed by the Minister of Health, the scale, as from August 1, 1962, being:

Senior Specialist, £2,480 to £2,800;
Junior Specialist, £1,980 to £2,340.

The conditions of appointment for the position and full information are available from the Secretary.

COOK HOSPITAL BOARD
GISBORNE

MEDICAL LABORATORY TECHNOLOGIST

APPLICATIONS are invited from suitably qualified persons for the position of Staff Medical Laboratory Technologist. The successful applicant will be expected to take a special interest in either the Department of Haematology or Bacteriology.

Applications, accompanied by two recent testimonials, should be addressed to the Medical Superintendent.

SOUTH OTAGO HOSPITAL BOARD
BALCLUTHA HOSPITAL

CHARGE BACTERIOLOGIST

Applications from either qualified medical laboratory technologists or candidates for the final qualifying examination in October are invited for the above position.

Salary payable in accordance with Grading under Hospital Employment Regulations.

A modern three-bedroomed house situated in the Hospital Grounds is available for a married appointee and single accommodation is also available.

Applications to be addressed to the Medical Superintendent, Hospital, Balclutha, from whom further particulars are available.

P. SHARPIN,
Managing Secretary.

Lactic Dehydrogenase and the Applications of its Estimation in the Routine Clinical Laboratory

T. E. BROWN, A.N.Z.I.M.L.T.
C/o Drs Perry and Fitzgerald, 685 George Street, Dunedin.*

(A paper read at the 1963 Annual Conference of the N.Z.I.M.L.T.)

Introduction

The diagnostic use of the estimation of lactic dehydrogenase (LDH) in serum for myocardial infarction; and in serum, cerebrospinal fluid, urine, gastric contents and serous effusions for malignancy, is now well established. The discovery of the five isoenzymes of LDH has now made this one of the most useful and specific enzyme estimations available in the routine clinical laboratory.

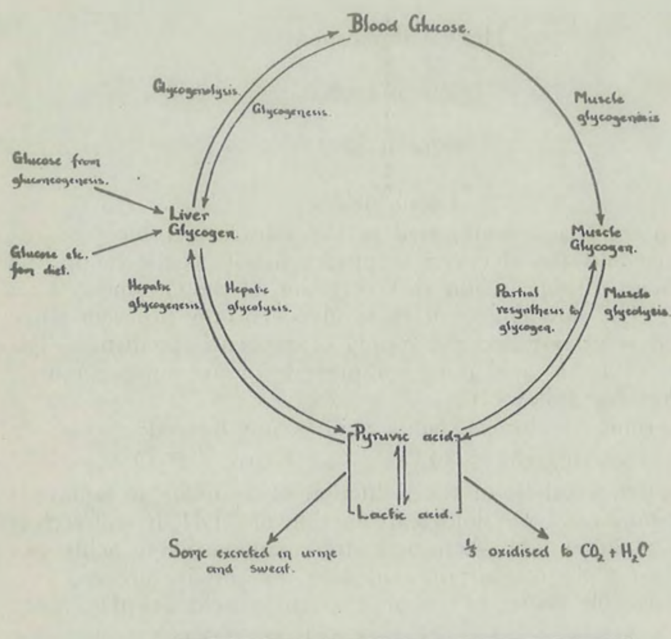
The purpose of this paper is to present a review of the literature on lactic dehydrogenase (LDH) and its isoenzymes, with reference to enzyme structure and mode of action, estimation and interpretation of the results obtained in various body fluids. The use of heat stability of the isoenzyme released from myocardial tissue following infarction (as distinct from the isoenzymes released from other tissue sources) and the isoenzyme pattern in other diseases, is also described with case results.

Biochemistry of LDH

LDH is a zinc-thiol containing protein, with a basic composition of 6 adenine, 5 thymine, 2 guanine and 2 cytosine molecules. It has a molecular weight of between 100,000 and 150,000 (estimated as 140,000 by Crockson[1], 1961). LDH is concerned with the final step in anaerobic glycolysis and catalyses the reduction of pyruvate to lactate. (See Figure 1).

A good physiological example of the reaction is shown when muscle contracts in a medium from which oxygen has been excluded. The glycogen is changed, in the presence of phosphate

*Author's present address: c/o Drs Taylor and Lycette, Munster Chambers, Tennyson St., Napier.



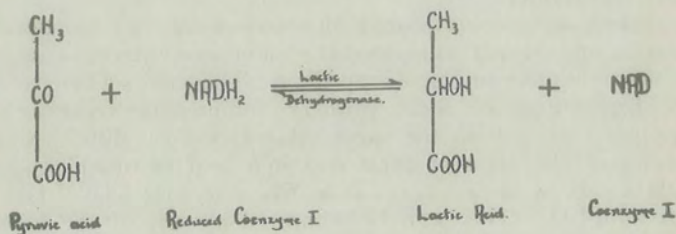
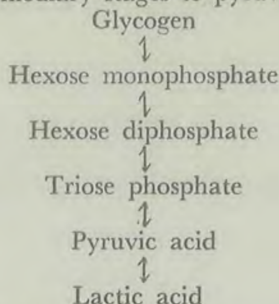


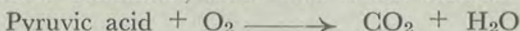
Fig. 1.

(adenosine di-phosphate and adenosine tri-phosphate), *via* the anaerobic glycolytic pathway, through a series of complex phosphorylated intermediary stages to pyruvic and lactic acids:—



When oxygen is readmitted to the muscle, aerobic recovery takes place and the glycogen reappears slowly as the lactic and pyruvic acids disappear, due to a reversion of the enzyme systems involved. The slow change back to glycogen only proceeds when the muscle is at rest and the supply of oxygen is plentiful. The formation of lactic acid does not precede or accompany muscle contraction, but follows it.

In aerobic conditions, lactic acid is not formed:



$$\text{Pyruvic acid} + O_2 \longrightarrow CO_2 + H_2O$$

Although catalysis of the reduction of pyruvate to lactate is the most important physiological function of LDH, it will reduce a variety of other alpha-keto and alpha-gamma-diketo acids; but only against alpha-oxobutyric acid does the activity proceed at a rate comparable with that against pyruvic acid at pH 7.8-8.0 Oxidation of lactic acid at this pH is very slow and the equilibrium of the reaction, pyruvate to lactate, lies in the formation of lactate. As the pH rises towards 9.5 activity against lactate increases, reaching a maximum at pH 9.5 where oxidation of lactate is favoured, reversing the equilibrium.

LDH Isoenzymes

When it was shown that all enzymes are not homogeneous, Markert and Moller[6] suggested the name isoenzymes, or isozymes, for the fractions which make up the total enzyme. These isozymes probably differ in their primary amino-acid sequence and, although they act on the same substrates, they differ from one another in the rates of their reactions and in other properties. LDH has been shown to occur as five isozymes: LD_1, LD_2, LD_3, LD_4 and LD_5. They can be separated by any of the following methods:—

(a) *Immunological*: Use of anti-LDx prepared in roosters and rabbits against tissue LDH prepared from homogenates. The

presence of more than one isozyme in tissue means that the results are not specific for one isozyme.

(b) *pH Optimum*: The five isozymes have different pH optima, ranging from 7.8 to 8.6.

(c) *Selective Inhibition*: By different ions, compounds or coenzyme analogues. (*e.g.*, LD_1 action against alpha-oxobutyric acid is less than 15% of the action exhibited by LD_5; the ability to use TPN instead of DPN as coenzyme also differs with each isozyme, as does the inhibiting action of oxalate and oxamate on the activity towards different substrates.)

(d) *Electrophoretic Mobility*: This has been the most widely used method of separation up to the present[9]. Acetyl cellulose foils, cellulose acetate strips, starch or agar gels and starch blocks have been used. The migration of the five isozymes is seen in Figure 2.

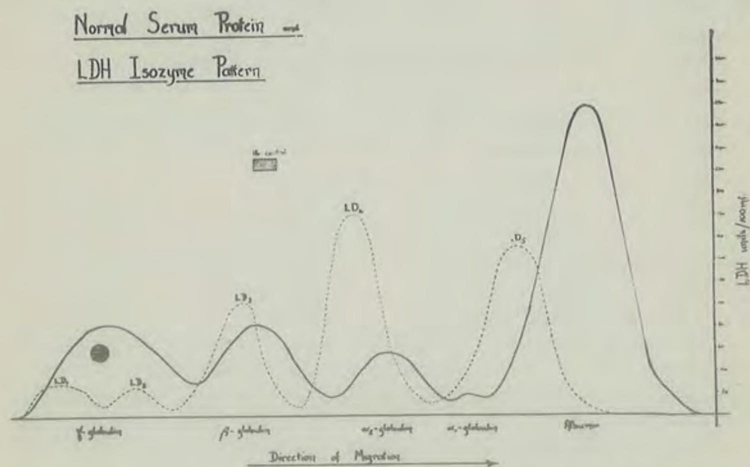


Fig. 2. Showing the electrophoretic mobility of the five LDH isozymes against the background of a normal serum protein tracing.

After separation, the strips are cut into fractions, and the enzyme is eluted into a buffer solution and estimated by one of the methods outlined in this paper.

(e) *Chromatographic Separation and Elution*: Carboxymethyl cellulose, dimethyl-aminoethyl cellulose (DEAE) and Sephadex DEAE have been used. Sephadex DEAE[7] is the newest of the resins to be employed for this purpose, and the method shows promise of being quick, simple and of giving quantitative

results. These qualities make it suitable for use in laboratories where the time factor is an important consideration.

The principle of the method is to salt out the globulins plus isozyme in an HCl-EDTA-triethanolamine HCl buffer with varying molarities of sodium chloride, and adsorbing the precipitate on to Sephadex. After centrifugation, the enzyme is estimated in the clear supernatant.

(f) *Heat Stability*: LD_5 is relatively heat stable and will withstand 65°C. for 30 minutes, but LD_1 is inactivated by 53°C. in six minutes. The temperature is critical and must be controlled to within ±1°C. of the required temperature for the measurement of the fractions. In normal serum, only 14% ± 5% of the total activity remains after incubation at 65°C. for thirty minutes. After infarction, up to 57% may remain.

The method is to dilute the serum 1 in 5 with water and then place a portion of it in a water-bath at 65°C. ± 1°C. for 30 minutes. LDH is then estimated on the heated and unheated specimens.

Estimation of LDH

After separation by one of the above methods, the activity of each of the isozyme fractions and the total LDH are estimated by one of the routine techniques, either manometric, spectrophotometric or colorimetric methods, the last two being the most popular.

Methods:

The manometric method of Green and Brosteaux[2] measures the oxygen uptake of the reaction, with methylene blue as the intermediate carrier between the reduced flavoprotein and molecular oxygen.

The spectrophotometric methods measure the rate of disappearance (with pyruvate substrate) or appearance (using a lactate substrate) of reduced coenzyme (DPNH) at 340mμ.

The colorimetric methods use two quite different principles:

(1) A colour change on the reduction of an oxidation-reduction dye (the most used of these dyes being the tetrazolium salts).[3]

(2) The decrease or increase of pyruvate concentration after the action of the enzyme on the substrate for a standard period of time, as measured by 2:4-dinitrophenylhydrazine.

The King method (1959)[4] using a lactate substrate at pH 10.0 and 2:4 dinitrophenylhydrazine to measure the increase in pyruvate, has been chosen by this laboratory, and the results presented in this paper were obtained by this technique.

Factors in favour of this method:

Stable reagents. The lactate buffer-substrate keeps indefinitely

in the refrigerator, whereas the pyruvate buffer-substrate has to be made up each six weeks. The coenzyme diphosphopyridine nucleotide (DPN) solution is stable in the freezer for six weeks, whereas the solution of its reduced form (DPNH) must be made up fresh for each batch of tests. It has also been noted that large errors can occur in reactions depending on the conversion of DPNH to DPN due to the development of unidentified inhibitors in the DPNH powder and solutions. The DPN is much less expensive than the DPNH, and is much easier to obtain because of its greater stability.

Ease of estimation :

(a) There is no need for any equipment that is not found in a routine laboratory. Any colorimeter is suitable, a spectrophotometer is not needed.

(b) The time factor, which is of primary importance in a busy laboratory. Actual working time is no more than a few minutes, even though the test takes 35 minutes to perform. (The bulk of this time is taken up in the incubation and colour development periods).

LDH is stable at room temperature for 3-4 days without any apparent loss of activity and, when frozen, the enzyme appears to be stable indefinitely. The urea concentration of the serum has been shown to cause denaturation of the enzyme when levels of 50-80 mg. per 100 ml. and over are reached.

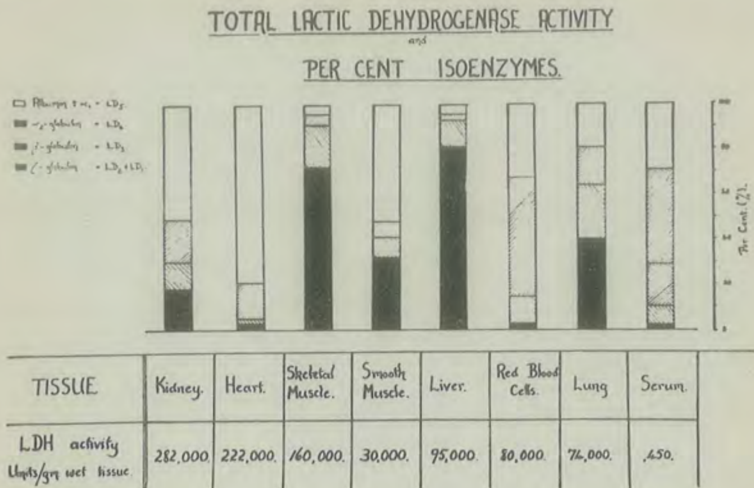
Haemolysis makes a specimen useless for the estimation, as the large amount of LDH present in the red blood cells may increase the serum value as much as 100-fold, and faint visual haemolysis will often cause an increase of 2-3 times the normal value of the specimen.

Factors affecting results :

Traces of sodium cyanide, adrenalin, amytal and zinc salts appear to increase the enzyme activity. Sodium fluoride and heparin seem to have no effect. Oxalate, oxamate, nicotinamide, EDTA, and the salts of heavy metals (*e.g.*, silver, mercury, iodine and excess zinc) show varying degrees of inhibition. Oxalate and oxamate appear to compete with the pyruvate in the forming of a tertiary complex with the LDH and DPNH. Borate and acid pH (below 6.0) show complete inhibition.

Tissue Distribution of LDH

High LDH activity is typical for tissues with a high glycolytic rate and high oxygen uptake; LD_5 and LD_4 usually predominate in the isozyme pattern.[13] Tissues in this group include: heart[14], brain, kidney, smooth muscle and red blood cells. The high value shown by red cells explains the error caused by haemolysis in the sample. (1 ml. of completely lysed cells has an activity ranging from 16,000 to 180,000 units, with an average of 34,000 units.)



TISSUE	Kidney.	Heart.	Skeletal Muscle.	Smooth Muscle.	Liver.	Red Blood Cells.	Lung	Serum.
LDH activity Units/gm wet tissue	282,000.	222,000.	160,000.	30,000.	95,000.	80,000.	76,000.	450.

Fig. 3. Showing the distribution of LDH activity in various tissues.

The exception to this typical tissue picture is skeletal muscle, which has a low oxygen uptake and a high activity. This is explained because in exercise, the oxygen supply is limited by haemodynamic factors and energy production is almost entirely anaerobic. LD_1 predominates in its isozyme pattern.

Lower activity is characterised by tissues with high aerobic glycolytic mechanism and include: liver, spleen, lung, pancreas, intestinal mucosa, lymph nodes and synovial cells. These tissues are associated with a predominance of LD_1, LD_2 and LD_3 in their isozyme patterns.

It is interesting to note that during normal pregnancy, the smooth muscle of the uterus changes both its total activity and its isozyme pattern to that of skeletal muscle. The activity and pattern revert to normal after parturition.

From the above it can be seen that the isozymes have a characteristic distribution in human tissues, showing individual quantitative patterns[10]. Following tissue damage, the enzyme released will enter the blood stream and alter the normal serum isozyme pattern. If quantitative evaluation of the isozymes present in serum are carried out, along with a total enzyme estimation, the affected tissue can be pinpointed, and an approximation of the damage assessed. If more than one tissue is involved, the results are not so easy to evaluate from the pattern alone, and the use of the heat-stability test may provide a quicker answer.

LDH and its Isozymes in Body Fluids

The following three mechanisms have been suggested to account for the alterations in LDH found in various body fluids:—

(i) Necrosis of tissue containing LDH results in the release of the intracellular enzymes, which find their way into the surrounding tissue fluid or into the blood-stream. This appears to be the explanation for the rise found in myocardial infarction, necrotising pancreatitis, renal infarction and skeletal muscle trauma.

(ii) Necrosis or inflammation of tissue may result in the release of intracellular enzymes, which are transiently excreted, secreted or otherwise handled abnormally. Elevations of LDH associated with hepatitis, glomerulonephritis and dermatomyositis may, in part, be explained by this section.

(iii) Malignant transformation of tissue results in the exudation of the intracellular enzymes during the abnormally rapid cellular growth and impaired respiration. The consequent lack of energy from this source is compensated for by an increase in anaerobic glycolysis. Examples of this are granulocytic leukaemia, disseminated lymphoma and carcinoma.

Estimation of LDH is now being used as a diagnostic aid in pleural or other effusions, cerebro-spinal fluid, urine, gastric contents and serum.

Serous Effusions:

The use of LDH estimation as an index of the presence of malignant neoplastic tissue in contact with or bathed in the fluid, has been confirmed by many authors. It is necessary, however, to perform both serum LDH and effusion LDH estimations to obtain diagnostic results. In non-neoplastic effusions, the ELDH value is lower than the SLDH, but in neoplastic fluids, SLDH is lower than that found in the effusion. The rise in LDH in the effusion is due to the contribution of enzyme to the surrounding medium by rapidly dividing malignant cells (red blood cells also have high LDH activity, but this is not transferred to the surrounding medium unless there is lysis). The fluids containing neoplastic cells have therefore been likened to cell cultures, and the graph as drawn by Wroblewski[11] (Figure 4) demonstrates the point.

The ELDH remains higher than the SLDH because the globulin fraction of the effusion does not readily exchange with the plasma constituent, and the LDH, being primarily a globulin component, remains in the fluid.

Isozymes LD_3 and LD_4 are raised greatly in neoplastic effusions.

The presence of haemolysis in the effusion (as may frequently occur) makes the specimen useless for LDH estimation.

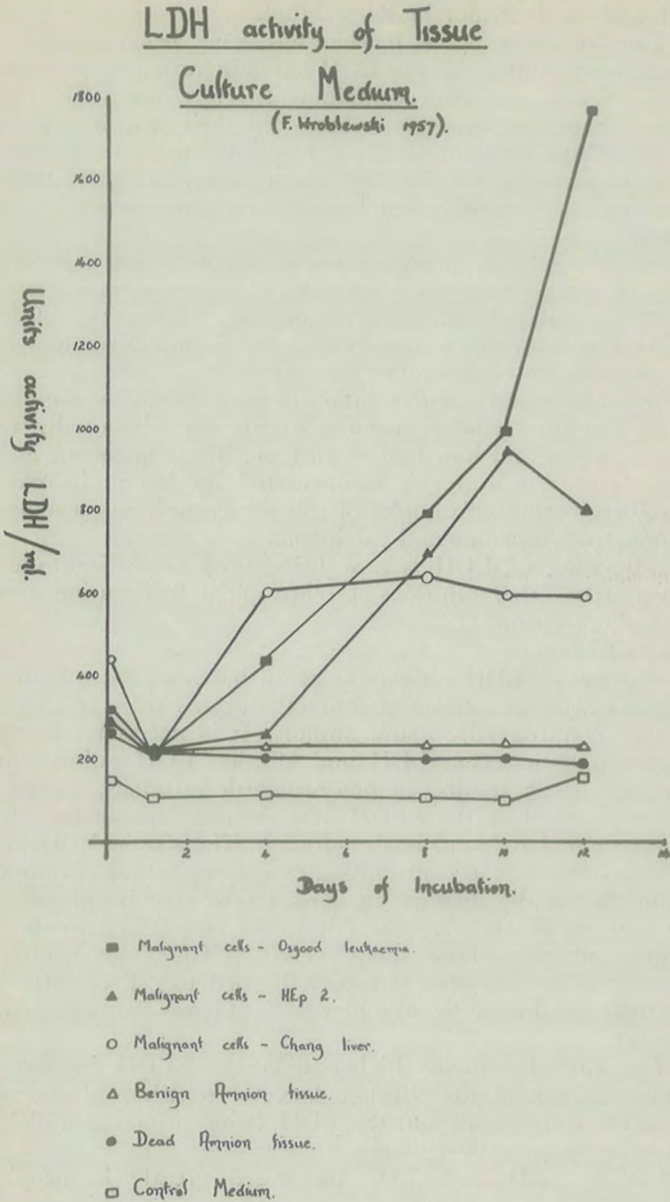


Fig. 4.

Some workers have declared that the estimation of LDH on effusions is valueless, on the grounds that they were unable to find any correlation between the cytological and enzymatic findings. There are, however, certain valid reasons to explain this lack of correlation:

Effusions coming into contact with malignant tissues have raised LDH levels, the degree of increment depending on the amount of growth coming into contact with the fluid. Cytologically there may be no malignant cells found if the growth is only in the tissue wall surrounding the fluid, but positive smears will always be found in cases in which the malignant cells are floating free in the effusion. In the first case the cytological result will be negative, with a positive LDH level; in the second both cytological and enzymatic evaluation will be positive.

If the patient is given treatment, the LDH level in the effusion may be found to be normal when the cytological findings are positive. This is because the therapy may slow the rate of metabolism and cell division, thus lowering the amount of enzyme exuded into the surrounding fluid, but not removing the cells present. (For example, the hormonal treatment of effusion associated with carcinoma of the breast. When the drugs are stopped, the ELDH level will again rise to an abnormal value. A similar reaction occurs in serum acid phosphatase levels in prostatic carcinoma treated with oestrogens.)

Positive LDH (in the absence of therapy) and cytological results are found in carcinoma of the lung, adenocarcinoma of the breast, duct cell carcinoma, and epidermoid carcinoma of the oesophagus. Negative LDH and cytology are found in pleural or abdominal effusion in nephrosis or cirrhosis, congestive heart failure and myxoedema.

Cerebro-Spinal Fluid :

Normal value: Less than 40 units per 100 ml.

The CSF LDH activity is independent of the serum LDH[8]. Increases are found with intracerebral or meningeal leukaemia, lymphosarcoma (Hodgkin's disease) and metastatic carcinoma showing central nervous system involvement. Untreated meningitis is also associated with a marked increase in CSF LDH and, on treatment, the LDH returns to normal, reflecting response. (This rise in LDH activity in untreated meningitis is due to the increased permeability of the blood-brain barrier, which permits entrance of plasma proteins, including LDH, along with leucocytes into the CSF.) The serum and CSF isozyme patterns are similar, so the passage of blood into the CSF does not alter the isozyme pattern noticeably, even though the total LDH is raised. During meningitis, however, the increase in isozyme LD_2, LD_3 and LD_4 is due to the lysis of the leucocytes in the CSF. As the leucocyte

count decreases, so does the LDH activity and the isozyme pattern returns to normal.

Urine:

Estimation of LDH in urine has been used as an indicator of malignant involvement of the urinary tract. The enzyme, being a protein of medium molecular weight, is handled by the kidney and excreted in the urine in the same way as other protein.

In 1961, Crockson's study[1] found no correlation between the red cell count and the ULDH in patients with haematuria or nephrotic syndrome, so neoplastic involvement of the urinary tract appears to be necessary for a raised value in urine.

The measurement of ULDH is carried out on a timed specimen of urine. The albumin clearance and the LDH clearance are then estimated:—

$$\frac{\text{LDH clearance}}{\text{Albumin clearance}} = 70\% \text{ (average) in normal individuals.}$$

Gastric Contents:

Normal value: Less than 39 units per mg. of soluble protein. (2% may have levels up to 55 units.)

After a modified augmented histamine test meal, a second dose of histamine is administered and 40-60 ml. of 2-5% sodium bicarbonate is installed into the stomach over a 10-12 minute period. This neutralises the acid which is secreted by the stimulated cells of the gastric mucosa and keeps the pH above 6.0. The stomach contents are partially aspirated each ten minutes, and the whole aspirate is collected through gauze into an ice-bath. Blood or bile-stained specimens are discarded.

LDH and protein are estimated. (The Biuret method is suitable for the estimation of protein.)

There is no correlation between the serum LDH and the gastric contents LDH[5].

70% of normal patients have values below 23 units per mg. of soluble protein.

68% of gastric ulcer patients have values below 39 units.

82% of duodenal ulcer patients have values below 36 units.

100% of gastric carcinoma patients have values above 55 units (range 55-87 units; average 71 units).

Serum:

Increased LDH values are found in myocardial infarction, acute hepatitis, muscular disorders (surgery and dystrophy), disseminated secondary carcinoma (especially if in the liver), granulocytic leukaemia, haemolytic disease of the newborn, haemolytic and megaloblastic anaemias. Some cases of acute pancreatitis, myositis, renal disease and normal pregnancies show a moderate increase.

Because of the increases found in many disorders, estimation of total LDH may be inconclusive, whereas the isozyme pattern will give precise and accurate results as to the tissue affected and the amount of damage.

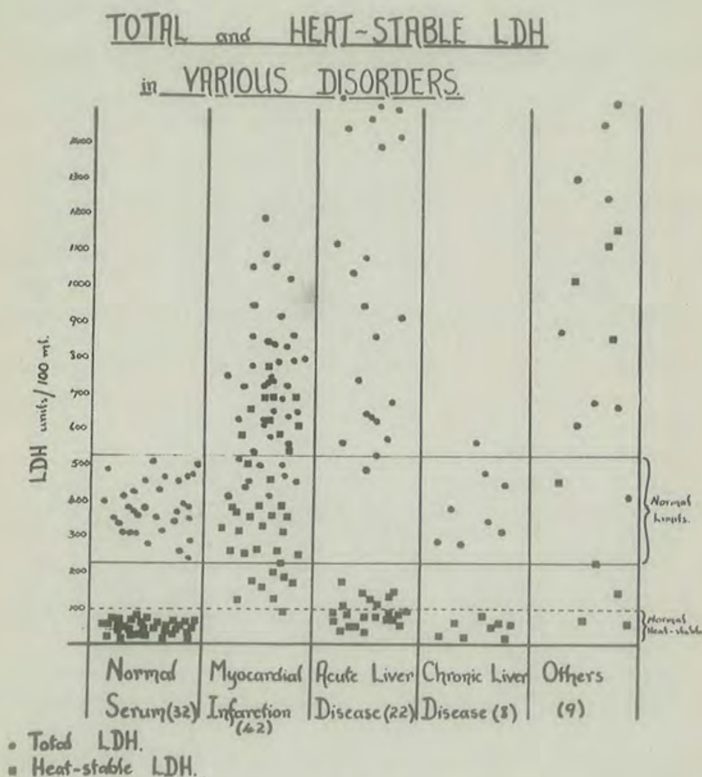


Fig. 5.

Figure 5 illustrates the total and heat-stable LDH values found in normal serum, and in serum from cases of myocardial infarction, acute and chronic liver disease.

Myocardial infarction. SLDH estimation is most useful when the electrocardiograph findings are equivocal owing to previous infarction, a bundle branch block, digitalis therapy and so on. Normal values are found in heart failure, pericarditis, coronary insufficiency and angina. The total LDH may rise as much as ten-fold in the first 12 hours following an infarct, reaching a peak at 24-48 hours, and returning to normal in 7-12 days follow-

ing infarction. The total LDH is not always raised in minor tissue damage, although a significant rise can be shown if multiple estimations are performed. The isozyme pattern will show a marked increase in LD_4 and LD_5 after any myocardial tissue damage, and the heat-stable value will be 50% or more when the total LDH is at peak value. This increase in LD_4 and LD_5 is accompanied by a relative and sometimes absolute decrease in the other three isozymes. In myocardial disorders the LD_5 level is always greater than the LD_4, the normal ratio of LD_4:LD_5 being 1.3:1 to 1.6:1. Following infarction, the ratio is approximately 0.7:1.

A rise is shown after pulmonary embolism (*i.e.*, a closure of a pulmonary artery or one of its branches by a clot resulting in pulmonary oedema or haemorrhagic shock), whereas the serum glutamic-oxalacetic transaminase (SGO-T) level remains normal.

The usefulness of the total LDH and heat-stability results in diagnosing myocardial infarcts, as distinct from other conditions, is clearly reflected in Figure 5. The amount of heat-stable enzyme left in the infarct cases, as compared with normal or hepatic disorders, shows the use of this simple method of detecting an increase in the LD_5 fraction.

Muscle Damage.

The SLDH level is raised following surgery and in muscular dystrophy; but not in muscular atrophy of neurological origin.

After surgery for mitral or pulmonary stenosis, and for resection of the stomach, 14 patients showed raised LDH and SGO-T levels within four hours of the operation. Normal values were regained post-operatively: on the fifth day for SGO-T and on the tenth to twelfth day for SLDH. If an infarct occurs within the first ten days following surgery, the estimation of SGO-T and/or total LDH is virtually useless, but by estimating the amount of LD_5 or heat-stable LDH present, it is possible for a diagnosis to be made. For example: A patient had chest pain and giddiness two days following an operation for partial gastrectomy. History of a previous infarct made the electrocardiograph findings inconclusive.

Enzyme estimations on the second day showed:—

SGO-T : 280 units per 100 ml.
LDH : 1,400 units per 100 ml.
Heat-stable LDH : 335 units per 100 ml. (or 24% of the total LDH).

On the third, fourth and tenth days:—

Third Day

SGO-T : 272 units per 100 ml.
LDH : 1,410 units per 100 ml.
Heat-stable LDH : 340 units per 100 ml. (24%).

Fourth Day

SGO-T	:	185 units per 100 ml.
LDH	:	1,385 units per 100 ml.
Heat-stable LDH	:	725 units per 100 ml. (53%).

Tenth Day

SGO-T	:	31 units per 100 ml.
LDH	:	680 units per 100 ml.
Heat-stable LDH	:	185 units per 100 ml. (29%).

Although no significant rise was evident in either enzyme (probably due to the loss of 'operation' enzyme as the gain in activity from the infarct proceeded), the rise in heat-stable activity up to 53% of the total LDH activity was quite diagnostic, because the heart is the only organ with sufficient LD_5 to affect the heat-stable fraction to this degree in the absence of massive haemolysis.

Only one case of muscular dystrophy was available for this study, and the LDH level, both total and heat-stable, were typical of a muscular disorder, showing low heat stability:

Total LDH	:	625 units per 100 ml.
Heat-stable LDH	:	95 units per 100 ml.

Liver Diseases.

Total LDH is usually substantially raised in the early stages of acute viral hepatitis (*i.e.,* during the initial inflamation and necrosis of the liver parenchyma), but the values do not follow the course of the disease as do the levels of SGO-T and SGP-T. Chronic and toxic jaundice, and infectious mononucleosis with liver involvement show normal or only slightly raised activities. Obstructive jaundice and cirrhosis show normal values. LD_1 is the isozyme fraction which is greatly raised in liver disorders and, being heat-labile, is completely destroyed at 65°C. in thirty minutes, so normal heat-stable values are obtained in hepatic disorders.

Leukaemia.

SLDH is raised in acute and chronic granulocytic leukaemia and in acute lymphoblastic leukaemia (but not in chronic lymphocytic leukaemia). Normal values are found in aleukaemic leukaemia. Serial enzyme studies reflect the course of the disease, falling in remissions and rising in relapses. In most cases, the LDH level parallels the white count but, occasionally, the enzyme level will foreshadow a relapse up to three weeks before any change in the white count is evident.

Summary

Methods of separation of lactic dehydrogenase isozymes and the estimation of total LDH are mentioned, with special reference to a simple heat-stability procedure which allows quick, easy separation of LD_5 from the other LDH isozymes.

Results obtained in various body fluids are reported, with a summary of the techniques required for the collection and performance of the estimation of LDH.

REFERENCES:

1. Crockson, J. (1961), *Lancet,* **i**, 140-142.
2. Green, D. E. and Brosteaux, J (1936), *Biochem. J.,* **30**, 1489.
3. Hsieh, K. M. and Blumenthal, H. T. (1956), *Proc. Soc. exp. Biol. Med.,* **91**, 626.
4. King, J. (1959), *J. med. Lab. Technol.,* **16**, 265.
5. Macoun, Mary L., Builder, Janet, E. and Piper, D. (1963), *Aust. Ann. Med.,* **12**, 160-165.
6. Merkert, C. L. and Moller, F. (1959), *Proc. Nat. Acad. Sci.,* **45**, 753.
7. Richterich, R. (1963), *Clin. chim. Acta,* **8**, 178-192.
8. van der Helm, H. G. (1963), *Ibid.,* **8**, 193-196.
9. Vessell, E. S. and Bearn, A. G. (1957), *Proc. Soc. exp. Biol. Med.,* **94**, 96-97.
10. Vessell, E. S. and Bearn, A. G. (1961), *J. clin. Invest.,* **40**, 586-591.
11. Wroblewski, F. (1958), *Scand. J. clin. Lab. Invest.,* **10**, Suppl. 31.
12. Wroblewski, F. (1957), *Amer. J. med. Sci.,* **234**, 301.
13. Wroblewski, F., Plageman, P. G. and Gregory, K. F. (1960), *J. biol. Chem.,* **235**, 2282-2293.
14. Wroblewski, F., Ross, C. and Gregory, K. (1960), *New Engl. J. Med.,* **263**, 531-536.

Since the reading of this paper at the N.Z.I.M.L.T. Conference in 1963, a paper confirming the findings on the heat-stability of LD has been published:

Bell, R. L. (1963), *Amer. J. clin. Path.,* **40**, 216-221.

Associateship Diplomas

Members of the Institute who hold the Certificate of Proficiency, or approved equivalent qualification, are reminded that they are entitled to apply for their diplomas as Associates of the Institute.

All applications should be addressed to the Secretary, and should include the applicant's full name and details of the qualification held, together with the year in which it was obtained.

A Micro Drop Technique for the Titration of Anti-Streptolysin O in Human Serum

B. F. DAWKINS, A.N.Z.I.M.L.T.
Department of Serology, Central Laboratory, Auckland Hospital.

(Received for publication May 1964.)

Following the micro modification of the staphylococcal anti-alpha-haemolysin test[2], a similar scheme was carried out to modify the anti-streptolysin 0 titre.

Using these micro techniques, it is possible to test for the above two antibodies with only 11 drops of serum (approximately 0.37 ml.) — an advantage when both tests are required simultaneously on children, and/or only a small amount of serum is available.

Various other methods for the anti-streptolysin 0 titre using micro quantities have been described in literature, *e.g.*, Crawford and Robinson (1954)[1], Goldin and Kaplan (1955)[3], Rappaport and Stark (1956)[6], and Jablon, Saul and Saslaw (1958)[4].

Materials

1. *Streptolysin 0 buffer.*

NaCl	7.4 g.
KH_2PO_4	3.17 g.
Na_2HPO_4	1.81 g.

Dissolve in 1,000 ml. of distilled water and adjust pH to 6.5 — 6.7 with NaOH solution. Store at 4°C.

2. *Red Cell Suspension.*

Human group 0 red cells from the blood bank are washed in saline until the supernatant is colourless. The final centrifugation should be at 2,000 r.p.m. for 15 minutes. Cells requiring more than five washings should be discarded. Make a 5% suspension of cells in streptolysin 0 buffer.

3. *Streptolysin 0 Reagent (lyophilized)*.*

Rehydrate immediately before use.

4. *Patient's Serum.*

Contaminated, chylous, or red-tinged specimens are unsatisfactory.

5. *Control Serum.*

Of known streptolysin 0 antibody strength. Stored at -20°C.

6. Pasteur pipettes calibrated to deliver 30 drops/ml. (Note: This calibration should be accurate.)

*Baltimore Biological Laboratory, Baltimore, Maryland, U.S.A.

	Serum Dilutions												Red Cell Control	Strepto-lysin Control
	1/10		1/100					1/500						
Tube Number	1	2	3	4	5	6	7	8	9	10	11	12		
drops Serum	8	2	10	8	6	4	3	10	8	6	4	2	—	—
drops Streptolysin 0 buffer	2	8	0	2	4	6	7	0	2	4	6	8	15	10
Shake gently to mix.														
drops Streptolysin 0 reagent	5	5	5	5	5	5	5	5	5	5	5	5	—	5
Shake gently to mix; Incubate at 37°C. for 15 minutes.														
drops of 5% red cell suspension	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Shake gently; Incubate at 37°C. for 45 minutes, shaking after first 15 minutes. Centrifuge tubes for 1 minute at 1,000-1,500 r.p.m.														
Unit value of each tube	12	50	100	125	166	250	333	500	625	833	1250	2500	Controls	

Table I

Methods

A. *Macro*. Basically that of Rantz and Randall (1945)[5], using 10 x 100 mm. test tubes.

The serum dilutions are made up to 1 ml. with buffer and 0.5 ml. of streptolysin 0 reagent is added. This is mixed gently and put in a 37°C. waterbath for 15 minutes. 0.5 ml. of freshly prepared 5% cell suspension is added, mixed gently and incubated for a further 45 minutes at 37°C., shaking after the first 15 minutes. Centrifuge the tubes for one minute at 1,000 - 1,500 r.p.m. to detect any haemolysis. Positive serum, streptolysin 0 reagent, and red blood cell controls are put up simultaneously; the positive serum being treated exactly as for a test serum.

The anti-streptolysin 0 titre, expressed in Todd units, is the reciprocal of the highest dilution of serum showing no haemolysis.

B. *Micro*. Using 8 x 75 mm. test tubes and a calibrated pasteur pipette. (The five drop quantities can be conveniently delivered using a 1 ml. pipette, with 0.167 ml. graduations.)
Serum dilutions:
1/10 = 0.1 ml. of serum + 0.9 ml. of buffer
1/100 = 0.2 ml. of 1/10 + 1.8 ml. of buffer
1/500 = 0.2 ml. of 1/100 + 0.8 ml. of buffer

Results

The limit of error for the anti-streptolysin 0 titre is quoted in literature to be ± 1 tube. Table II, then, indicates that all of

MACRO TITRE	MICRO TITRE		
	Same	1 tube higher	1 tube lower
12 or less	13	2	—
50	13	1	1
100	13	1	1
125	13	1	1
166	14	—	1
250	14	1	—
333	9	—	1
500	9	1	—
625	10	—	—
Total	108	7	5
Percentage	90	5.8	4.2

Table II

the 120 comparative macro and micro samples agreed, 108 (90%) of the tests showing identical titres by both techniques and 12 differing by only one tube.

Conclusion

120 sera were tested for anti-streptolysin 0 using the standard macro and a micro technique simultaneously. The macro and micro titres did not appear to vary significantly in any serum tested, though greater care is obviously needed in performing the micro test in order to achieve accuracy.

Two factors which appeared to affect both methods equally were noted. Freezing and thawing more than once was found to reduce a high titre considerably, while different batches of red blood cells occasionally gave differing results on the same serum.

Acknowledgment

The author wishes to thank Mr A. Fischman for an objective criticism of this paper.

REFERENCES:

1. Crawford, Y. E. and Robinson, J. J. (1954), *Amer. J. clin. Path.*, **24**, 1103.
2. Dawkins, B. F. (1964), *N.Z. J. med. Lab. Technol.*, **18**, 55.
3. Goldin, M. and Kaplan, M. A. (1955), *Amer. J. clin. Path.*, **25**, 1432.
4. Jablon, J. M., Saul, M. and Saslaw, M. S. (1958), *Ibid.*, **30**, 83.
5. Rantz, L. A. and Randall, E. (1945), *Proc. Soc. exp. Biol. Med.*, **59**, 22.
6. Rappaport, F. and Stark, G. J. (1956), *Amer. J. med. Technol.*, **22**, 103.

Annual Subscriptions

The Treasurer would be grateful if members who have not paid their 1964/65 subscriptions would kindly forward their remittances as soon as possible.

These subscriptions became due on April 1, 1964 and in accordance with the terms of Rule 10(c) members who are more than six months in arrears become liable to forfeit their rights as members.

An Improved Colorimetric Method for the Estimation of Lipase in Serum Using Phenyl Laurate as a Substrate

A. G. WILSON
Chemical Pathology Laboratory, Pathology Department, Medical School, Dunedin.

(Winner in the Technical Section of the Junior Essay Competition 1964.)

The lipases are classified with the esterases, several types of which are present in serum. The two of most significance are esterase, which acts on short chain fatty acids, and lipase which acts on long chain fatty acids containing 8 to 18 carbon atoms. The lipases hydrolyse fats to form end products of glycerol and fatty acids. The enzyme is water soluble, presumably a protein, and is very unstable, being easily rendered inert by acid.

The pancreatic lipase is undoubtedly the most important fat splitting enzyme in the digestive tract. Its activity is dependent on the surface area of fat available. The presence of bile salts in the intestine accelerates lipase activity due to the physicochemical action of the salts in facilitating closer contact between the water soluble lipase and the fat globule.

The concentration of pancreatic enzymes in normal serum is relatively low. In acute pancreatitis the swelling of the acinar cells may lead to regurgitation of the enzymes into the blood stream. The diagnostic tests commonly employed are the estimation of amylase and lipase activity. The results of the two tests do not always parallel one another. Cases have been described by various authors that show the estimation of serum lipase in the diagnosis of acute pancreatitis to be more specific. In a typical case it has been found that the lipase remains elevated for a longer period than the amylase and that recurring attacks of pain show an immediate increase in lipase, whereas the amylase shows little or no increase.

There are a number of methods available for the estimation of lipase, none of which is entirely satisfactory or specific for lipase of pancreatic origin.

The method of Cherry and Crandall (1932)[1], and its subsequent modification, utilises olive oil as substrate. Various workers have found that different samples of olive oil vary in their rate of hydrolysis. Also, the substrate is not specific, as esterases hydrolyse the olive oil to some extent. The method is not satisfactory in that a relatively large amount of serum is required (2 ml.) and there is a long incubation period (24 hours). The titrations are laborious and the endpoint is difficult to determine.

Tietz *et al.* (1959)[5] described a method with a modified Cherry and Crandall substrate. The incubation time was reduced

to six hours, but since the results were about half that of the original Cherry and Crandall method, there may be a greater error in the titrations. These workers stressed the importance of a buffer with an optimum pH and stated that the optimum pH of pancreatitis lipase is the same as the lipase of normal persons.

Vogel and Zieve (1963)[6], however, claim that serum of patients with acute pancreatitis contains a lipase which has a different optimum pH from that of normal persons. Their rapid turbidimetric method is claimed to be specific for pancreatitis lipase. They use a purified olive oil substrate which takes some time to prepare and must be used the same day. The incubation time is twenty minutes. The difference in optical density between zero time and the degree of clearing which has taken place, is described as a lipase unit. One unit is defined as a difference of 0.001 optical density. The method requires exacting conditions and extreme accuracy as the normal range is not more than 15 units. (*i.e.,* change in optical density of 0.015.)

A number of other methods are described using, as substrates: Tween 20, tributyrin, and alpha- and beta-naphthyl laurate. Most of the methods using these substrates have been criticised as being non-specific. For example, tributyrin was found to be hydrolysed by a pseudocholinesterase. The addition of specific activators for lipase and inhibitors for other enzymes has brought much criticism.

Perhaps, because of the conflicting results and technical difficulties associated with many of the methods suggested, the original method of Cherry and Crandall has persisted in most laboratories.

One substrate that seemed worth further studies was phenyl laurate. Techniques published using this include that of Radercht and Moskau (1959)[3] and the modification by Saifer and Perle (1961)[4].

The substrate phenyl laurate is a long chain fatty acid containing 12 carbon atoms, with a phenyl group attached. This is claimed to be split specifically by serum and pancreatic lipase. The phenol released is then measured simply and colorimetrically by the reaction with Folin and Ciocalteu's phenol reagent.

The addition of sodium cholate provides the physicochemical conditions to activate the lipase and also serves to inhibit the action of esterases on the substrate. Raderacht and Moskau (1959)[3], in their experiments, found that the optimum concentration was about 100 mg. per test and this concentration resulted in a 300% increase in activity over that where no activator was used.

The optimum pH for the method is 7.4.

Saifer and Perle showed that pseudocholinesterase has no effect on the substrate.

The method as described by Saifer and Perle, however, seemed unnecessarily complicated and a number of modifications were made so that technically the test closely parallels the King and Armstrong[2] method for phosphatase estimations. However, the relative concentrations of serum, substrate, buffer and activator are the same as suggested by Saifer and Perle.

'Tris' buffer has been substituted for veronal acetate buffer suggested by Saifer and Perle.

Materials

1. Phenyl laurate (Eastman Organic Chemicals, Rochester, New York).

This is kept in a refrigerator at -20°C.

Dissolve 250 mg. of phenyl laurate in 25 ml. of acetone.

Store in a refrigerator at 4°C. (Keeps for several months.)

2. Tris (hydroxymethyl) aminomethone buffer. pH 7.4. 0.2 molar.

Dissolve 24.2 g. of tris compound in about 500 ml. distilled water. Add 170ml. of N hydrochloric acid and make up a litre with distilled water. Check the pH before use.

3. Sodium cholate (Mann Research Laboratories, New York).

A 3.3 g./100 ml. solution in distilled water. It is convenient to make 500 ml. at a time.

4. Folin and Ciocalteu's Reagent.

Prepared as described in *J. biol. Chem.* (1927), 73, 267.

This is diluted 1 in 3 with distilled water before use.

5. Sodium carbonate anhydrous (15% w/v).

75 g. are dissolved in distilled water and made up to 500 ml.

6. Stock standard phenol (1 mg./ml.).

1 g. of pure crystalline phenol is dissolved in and made up with 1 litre of 0.1 N hydrochloric acid.

7. Stock standard phenol and reagent.

5ml. of the stock standard is accurately measured into a 500 ml. volumetric flask. 100 ml. of the diluted Folin and Ciocalteu's reagent is added and made up to the mark with water. One ml. contains 0.01 mg.

This solution will keep at least six months in the refrigerator.

It will be noticed that the Folin and Ciocalteu's reagent, the sodium carbonate solution, and the standard phenol and reagent, are the same as that used in the King and Armstrong method for phosphatase estimation.

8. Substrate mixture.

This is prepared fresh for each series of tests. Add in order and mix by swirling to obtain a colloidal solution:

70 ml. of 3.3% sodium cholate solution.

20 ml. of Tris buffer.

2.5 ml. of phenyl laurate.

Method

Into two 12 mm. x 100 mm. tubes, one labelled 'test,' the other labelled 'blank,' pipette 4 ml. of the buffer substrate solution.

Incubate the tubes in a 37°C. water bath for three minutes to equilibrate, then add 0.2 ml. of serum to the 'test.' Leave for exactly 30 minutes, then add 1.8 ml. of the diluted Folin and Ciocalteu's reagent. Mix by inverting several times and stand at room temperature for 10 minutes.

1.8 ml. of the Folin and Ciocalteu's reagent is now added to the 'blank,' followed by 0.2 ml. of serum. Thereafter, the procedure is as the 'test.'

Centrifuge at 2,000 r.p.m. for 10 minutes.

It is necessary to use a 'swing out' type centrifuge, as the deposit is fine and should be packed at the bottom of the tube.

To 4 ml. of the clear supernatant add 2 ml. of 15% sodium carbonate solution. Mix and incubate at 37°C. for 10 minutes.

Standard :

4 ml. of the stock standard phenol and reagent are treated as a test supernatant.

Reagent Blank :

0.8 ml. of the dilute Folin and Ciocalteu's reagent is added to 3.2 ml. of water.

This is treated as a test supernatant.

Read in a Spekker absorptiometer, using filter No. 8, and a 1 cm. cuvette against water.

Calculation :

$$\frac{\text{Extinction of 'test'} - \text{Extinction of 'blank'}}{\text{Extinction of Standard} - \text{Reagent Blank}} \times 0.04 \times \frac{100}{0.133}$$

$$\frac{T}{S} \times 30 = \text{lipase units/100 ml. of serum.}$$

Normal Range : 2.5 to 5.6 units/100 ml.

Experimental

A large number of sera were tested from hospital patients with no liver, pancreatic or intestinal condition. The result fell within the normal limits as described by Saifer and Perle.

Pancreatic lipase was obtained in the form of a preparation of emulsified pancreas, and serial estimations were determined from which a standard deviation of 0.844 was calculated.

Serial dilutions of the pancreatic extract were tested and demonstrated that optical density versus lipase activity is linear to at least 20 units. This is shown on Figure 1.

Other sources of elevated lipase were obtained from Warner Chilcott Versatol E serum and from a patient with acute pancreatitis. (Serum amylase: 1,800 Somogyi units/100 ml.) These

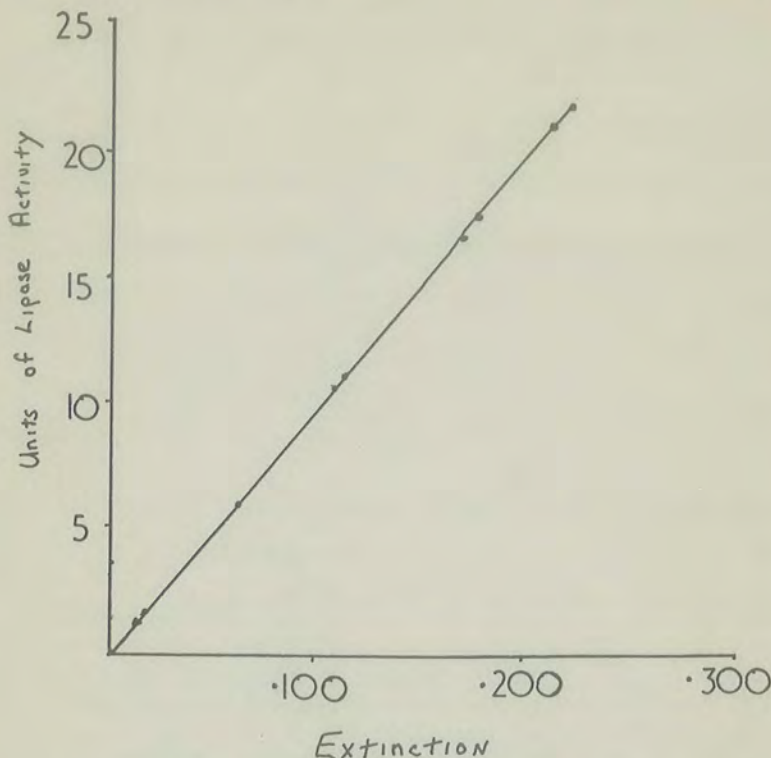


Fig. 1.

were compared in parallel with the technique of Cherry and Crandall.

They are shown on Table I.

The Cherry Crandall value for the Versatol E serum is stated to be 4.1 units/ml.

Repeated estimations on this serum by the phenyl laurate method failed to demonstrate an abnormal value. It is suggested that since the phenyl laurate method measures pancreatic lipase specifically, the abnormal value obtained with the Cherry Crandall technique was not due to lipase.

It can be seen that analysis of the pancreatic extract showed only a slightly elevated level by the Cherry Crandall technique but a significant increase by the phenyl laurate method.

This demonstrates that the latter method is more sensitive to pancreatic lipase.

The results for the patient with acute pancreatitis showed a marked increase by both methods. The Cherry Crandall value is probably due to hydrolysis by esterase in addition to lipase.

	SOURCE	Cherry Crandall	Phenyl Laurate
1.	Pancreatic extract	2.2 units/ml.	13.6 units/100 ml.
2.	Pancreatic extract	2.2 units/ml.	14.5 units/100 ml.
3.	Patient with acute pancreatitis	14.5 units/ml.	14.4 units/100 ml.
4.	Versatol E. Serum	3.9 units/ml.	4.4 units/100 ml.
5.	Versatol E. Serum	4.3 units/ml.	

Table I.

It is concluded that a raised lipase by the phenyl laurate technique is clinically more significant than that of Cherry Crandall.

Experiments using phenyl laurate twice and four times the concentration showed no significant change in activity.

Three buffers: veronal-acetate, Tris, and phosphate, all pH 7.4 and molarity 0.2 were compared. A series of tests with the pancreatic extract and the Versatol E serum gave no significant difference. Tris buffer was chosen because of its ease in preparation and stability on storage.

Serum which has been frozen will retain its lipolytic activity for up to three weeks.

In a small series it was found that ethylene diamine tetra acetic acid (EDTA) did not appear to affect the results.

Summary

A technique is described for the estimation of serum and pancreatic lipase which will split specifically a phenyl laurate substrate. The liberated phenol is estimated by a technique closely resembling the King and Armstrong phosphatase method.

Acknowledgment

The author is indebted to the helpful advice of Mr J. L. Braidwood.

REFERENCES:

1. Cherry, I. S. and Crandall, L. A. (1932), *Amer. J. Physiol.,* **100**, 266.
2. King, E. J. and Armstrong, A. R. (1934), *Canad. med. Ass. J.,* **31**, 376.
3. Raderecht, H. J. and Moskau, H. (1957), *Clin. chim. Acta.,* **4**, 221.
4. Saifer, A. and Perle, G. (1961), *Clin. Chem.,* **7**, 178.
5. Tietz, N. W., Borden, T. and Stepleton, J. D. (1959), *Amer. J. clin. Path.,* **31**, 148.
6. Vogel, W. C. and Zieve, L. (1963), *Clin. Chem.,* **9**, 168.

Accuracy and Quality Control in Clinical Chemistry

ADRIENNE RAMSAY

Laboratory, Public Hospital, Oamaru, North Otago.

(Winner in the Essay Section of the Junior Essay Competition 1964.)

Results of quantitative laboratory analyses are used by physicians to determine the clinical condition of patients and to follow their progress under medical therapy. Such results must be precise and accurate or they may be seriously misleading.

Reproducibility requires that repeated analyses give closely comparable results. Accuracy requires that results are not only reproducible, but also correct. The degree of reproducibility of a particular determination in daily routine work is affected by such variables as reliability of method and reagents; skill of person performing the test; accuracy and cleanliness of pipettes, glassware and cuvettes; calibration of colorimeter or spectrophotometer; and the method used for calculating results. Accuracy is also affected by these variables; and often, because reference standards and curves control only the last stages of an analysis, it is possible to get the value of the standard and instrument calibration correct, yet get an inaccurate value for the unknown.

It is, therefore, most necessary to have some means of determining the accuracy and precision of all laboratory analyses. This may be done by running a known control and standard with every test, and by setting up a quality control system. The object is to reject any batch of results where it is obvious that an error greater than that permitted has occurred.

In order to control all the steps of a procedure, it is necessary to use a known control, together with a known standard. A standard is a solution of known concentration of the constituent being determined. A stock solution is prepared by weight and diluted for use. A measured amount of the diluted stock solution takes part in the test and the true value is readily calculated. The final colour of the standard is used as a reference for calculating the values for the unknowns, and checks variations in instruments and reagents. However, in many procedures, the standard enters into the last stages only. Also, the standard does not resemble the blood specimen with its many varied constituents, some of which occasionally interfere with colour development. This is exemplified in the estimation of cholesterol, where excess bilirubin present develops a colour, giving a higher result. Thus, it is evident that the standard alone cannot serve as a check on the entire technique.

A control contains a known concentration of the constituent being determined as well as other constituents present in blood, and is used simultaneously with the unknown specimen. Since it goes through the same dilutions, protein precipitation and other steps as the unknown, it serves as a check on procedure, reagents, technique and instrument calibrations. Such a control may be prepared by pooling human sera and, by repeated analyses, determining values for the constituents. When deep frozen these controls are reasonably stable, though bacterial contamination sometimes occurs, and glucose and enzyme values decline. Commercially prepared, standardised, freeze-dried, pooled human serum is available as an alternative. This is best reconstituted immediately prior to use. A 'day to day' system of control may be carried out by first calculating the values of a set of unknown test sera using a known control, then using one of the now known tests to act as a control for the next day's test. This system has obvious limitations.

When the value of the known control does not check when calculated against the standard, it indicates that some factor in the procedure or instrument is not functioning properly.

Values of unknown constituents in a sample specimen may be calculated in various ways, some of which are more accurate than others. Linear, or semi-log graph sheets or optical density values may be used when methods follow Beer's law *(e.g.* glucose estimation). For methods which do not follow Beer's law (*e.g.*, colorimetric determination of transaminase) values of reference standards are plotted on standard semi-log graph sheets. Calculation of results using standards run with each determination has greater accuracy than calculation by precalibrated curves.

Where visual colorimeters are used, with a suitable standard:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown.}} \times \text{Concentration of standard} = \text{concentration of unknown.}$$

Using the optical density scale of a photoelectric colorimeter:

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times \text{Concentration of standard} = \text{concentration of unknown.}$$

These results can be quickly and accurately computed with the use of a slide rule.

Precalibrated curves tend to be unreliable because of the fact that colour development is influenced by time, temperature, new reagents and many other factors. If photoelectric colorimeters are not recalibrated occasionally to compensate for any change caused

by general wear and changing of physical constants, or curves are not checked with a standard, incorrect values for the unknowns will be obtained. Where calibration curves are necessary (*e.g.*, in the estimation of blood urea nitrogen by the urease method), two standards run with each determination are required for accuracy of results.

Quality control is a statistical system for measuring the degree of precision in clinical chemistry procedures, and does not measure the accuracy of a determination. It takes into consideration all the variables which are inherent in chemical determina-

Nº of TESTS	OD	Mgms	Difference from average	Difference Squared
1	445	123	17	289
2	370	103	3	9
3	370	103	3	9
4	398	110	4	16
5	385	107	1	1
6	398	110	4	16
7	385	107	1	1
8	435	121	15	225
9	375	105	1	1
10	405	112	6	36
11	400	111	5	25
12	405	112	6	36
13	385	107	1	1
14	375	105	1	1
15	360	100	6	36
16	410	114	8	64
17	360	100	6	36
18	375	105	1	1
19	370	103	3	9
20	405	112	6	36
21	425	118	12	144
22	365	101	5	25
23	422	117	11	121
24	380	106	0	0
25	400	111	5	25
26	355	99	7	49
27	385	107	1	1
28	398	110	4	16
29	422	117	11	121
30	375	105	1	1
31	398	110	4	16
32	355	99	7	49
33	385	107	1	1
34	405	112	6	36
35	365	101	5	25
36	405	112	6	36
37	385	107	1	1
38	398	110	4	16
39	405	112	6	36
40	368	102	4	16
		TOTAL 4233	TOTAL	1483
		AVERAGE 106 *	DIVIDED BY 39 ≏ 38 *	
			SD = $\sqrt{38}$ ≏ 6 *	

* ROUNDED to NEAREST WHOLE NUMBER

Table I. A calculation of Standard Deviation (S.D.) from the results of forty Nelson-Somogyi glucose estimations.

tions and which can effect the degree of reproducibility of a particular determination in daily routine work, as well as reflecting the effect of these variables in the magnitude of the 'range of allowable variation,' or 'confidence limit.' To establish such a range the standard deviation must first be calculated. Standard deviation is a mathematical measurement of the dispersion of values in a test series on either side of the average value, and is calculated after performing a series of tests under conditions as nearly identical as possible. The test values are recorded and from them a mean value is established. The difference of each value from the mean value is obtained and each is squared. All the squared values are added and the total is divided by the number of determinations minus one. The square root of this value is taken as one Standard Deviation. Table I gives an example. A limit based on three times the standard deviation is the one usually maintained. Results outside these limits should occur by chance in less than 1% of instances. The permitted variations naturally vary considerably for different blood constituents and are smaller (no greater than ± 3%) for inorganic substances than for organic substances (may be up to ± 10%).

The values ± 1, 2 and 3 S.D. can be applied graphically to prepare a quality control chart, as illustrated in Fig. 1. By plotting

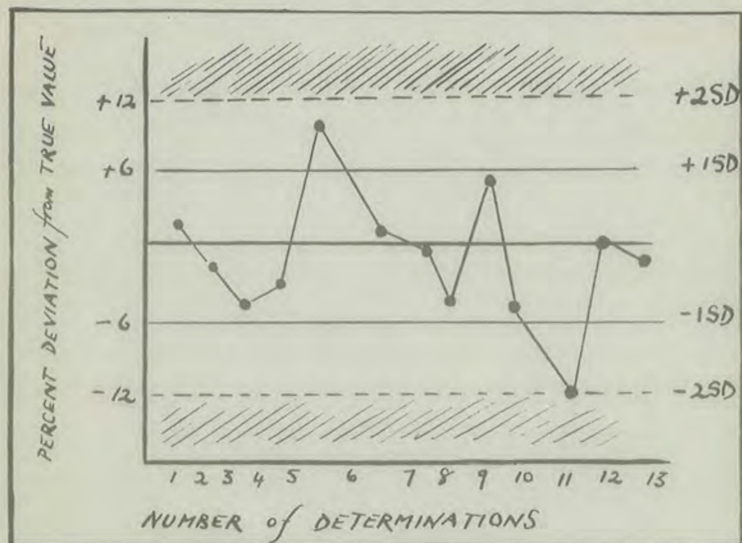


Figure 1. Specimen control chart for glucose determinations.

the daily values obtained for the control serum any fluctuations in test results will become immediately apparent. When test

values for the control fall within the range of ± 3 S.D., it indicates that the test procedure is functioning normally and the values are within the confidence limits. If this is not the case, it can be assumed that an avoidable error has occurred, and all analyses in that particular batch should be repeated. If, on repeating the control determination, results continue to fall outside the limit of error, investigation is needed to discover the cause.

There are many potential sources of error occurring in the individual specimen itself: the choice (whole blood, plasma or serum); condition (freedom from haemolysis or lipaemia); method of anticoagulation, if any; and stability; none of which can be adequately checked by a control. However, providing specimens are collected and stored under standardised conditions, such errors can be reduced to a minimum and, together with the maintenance of an adequate system of control, all results can be reported with complete confidence.

Commencing in 1965, the JOURNAL will be published in MARCH, JULY and NOVEMBER. Deadlines for copy for these issues will be February 1, June 1 and October 1 respectively.

Changes of Address

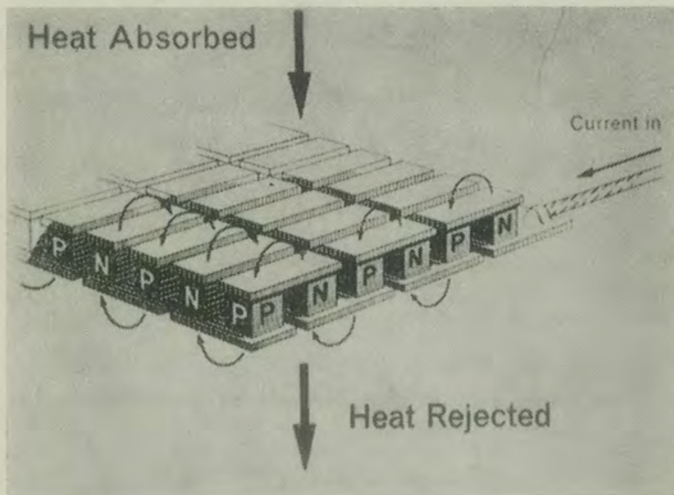
Members of the Institute and subscribers to the JOURNAL are asked to ensure minimal misdirection of correspondence by notifying any changes of addresses promptly to the Editor.

In the case of members, such notification will automatically ensure the registration of their new addresses in the official records of the Institute.

Thermo-Electric Cooling for Research, Hospitals and Industrial Laboratories

New cooling methods in spectrometry, surgery, microscopy, electronics.

More and more applications are being found for thermo-electric cooling modules, which use an electric current to pump heat from one face of a semi-conductor block to another (Fig. 1). The flow of heat may be regulated by reducing or reversing the current. Unlike traditional refrigerators, these modules have no moving parts, are completely silent in operation and contain no corrosive fluids; they therefore need no servicing. Perhaps their most striking quality, however, is their small size: a module



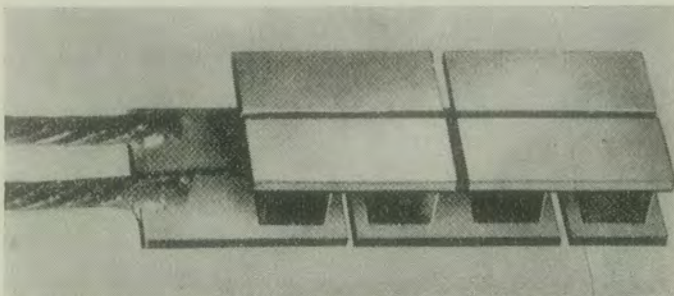


Fig. 1. TOP: Diagram illustrating the principle of thermo-electric cooling.
BOTTOM: A typical *Frigistor* thermo-electric cooling module.

capable of pumping 71 BTUs per hour is little larger than a matchbox.

Typical applications include cold stages for microscopes; reference chambers for rapid and accurate calibration of thermocouples; cooling of electronic apparatus (for example, photomultiplier tubes, infra-red detectors) to reduce thermal noise to a minimum; hygrometers; stabilisation of quartz oscillator crystals used in radar equipment; determinations of osmolarity by the depression of freezing point method; cooling of sample tables for use in neutron diffraction spectrometry; control of vapour in vacuum pumps.

In medicine, thermo-electric modules are used to cool the knives and stages of microtomes and are incorporated in freeze driers and tissue embedding apparatus for biological specimens. In addition, thermo-electric cooling may prove extremely valuable in open-heart surgery as a means of controlling hypothermia and subsequent reheating; and in artificial kidney operations to monitor the osmolarity of body fluids.

Construction and Operation

Thermo-electric cooling depends on the Peltier effect, whereby an electric current, passed through dissimilar semi-conductor blocks arranged alternately in series, causes heat to be absorbed at one set of alternate junctions and rejected at the other, the whole unit acting as a form of heat pump. One of the semiconducting materials has an excess of free electrons, the other has a deficiency of electrons. Passing a current forces some electrons from a state of low potential energy to one of high potential energy, so that heat must be taken in from the surroundings by the law of conservation of energy; and other electrons undergo the opposite process with an emission of heat.

Frigistor modules consist of 4, 6, 8 or 12 pairs of semiconductor blocks, and they accept a maximum direct current of 15, 30 or 60 amperes. The blocks (which are made from two different alloys of bismuth telluride with antimony and selenium) are joined by copper bridges and arranged with all the hot junctions on one side and all the cold junctions on the other. One side of the module is in contact with the area to be cooled or heated; the other is kept at a constant temperature by means of a heat sink.

Extremely rapid cooling of small components can be achieved. For example, when Frigistor modules are fitted to the stage and knife of a microtome for cutting biological specimens, the stage takes only one minute to reach –30°C from room temperature and the 160mm wedge knife reaches –20°C. in five minutes (Fig.2).

Two and Three-Stage Elements

The maximum temperature difference obtainable between

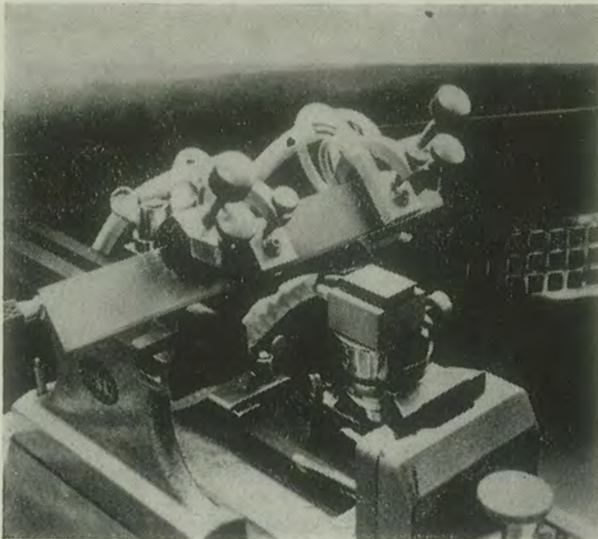


Fig. 2. *Frigistor* thermo-electric cooling modules fitted to a microtome for cutting biological specimens.

the faces of a standard module is 63°C. (when the hot junction temperature is 27°C.), but even greater temperature differences can be obtained by using the cold face of one element to cool a heat sink attached to the hot face of another. Temperatures of –90°C. to 100°C. can be achieved by three-stage thermo-electric coolers.

A two-stage Frigistor element is used by Edwards High Vacuum Ltd., of Crawley, in their new *Speedivac-Pearse* freeze drier for biological specimens (Fig. 3). It takes only five

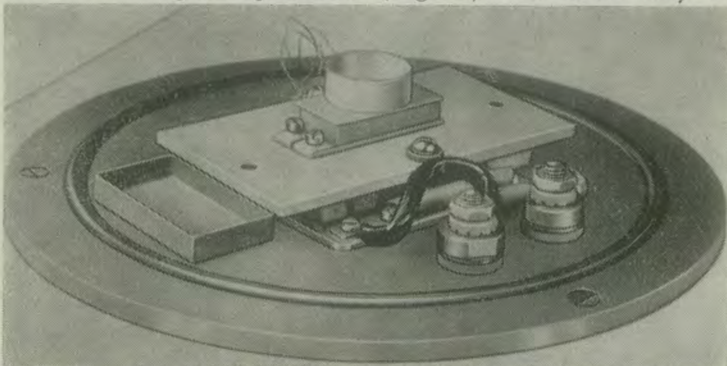


Fig. 3. Baseplate of the new *Speedivac-Pearse* freeze drier for biological specimens showing the two-stage Frigistor thermo-electric cooling element on which the specimen is placed under vacuum.

minutes after switching on the current at 25 amps to cool the platform from room temperature to –60°C.

Large Refrigeration and Air-Conditioning Installations

The largest cooling module available from De La Rue Frigistor Ltd. at present has a maximum current rating of 60 amps and will pump heat at a rate of some 140 BTUs per hour. However, the advantages of thermo-electricity — the silent operation, lack of servicing and savings of space and weight— make it eminently suitable for large refrigerators and air-conditioning systems.

At the moment thermo-electricity is more expensive than conventional means of refrigeration for domestic units above 2 cubic feet capacity, but this is due rather to the small volume of present-day production than to any innate costliness of the materials. At De La Rue Frigistor, for example, processes such as soldering are being automated as demand increases, and the price of Frigistor modules has recently been halved.

Further information from: *De La Rue Frigistor S.A.*, Caisse Postale 171, Fribourg, Switzerland; or from the local agents: Hoffman Ltd., P.O. Box 171, Dunedin.

Material supplied, free of copyright, by Engineering in Britain, *12 Swallow Street, London, W.1.*

Selected Abstracts

Contributors to this issue: R. D. Allan, J. Case, E. K. Fletcher, B. Glynn-Jones, J. Rees, H. C. W. Shott, D. Tingle.

BLOOD BANKING

Hemolytic Disease of the Newborn Caused by Anti-Jk^b. Wagman, E. and Bove, J. R. (1964), *Amer. J. clin. Path.*, **41**, 481.

A mild case of haemolytic disease is described, which results from a maternal antibody identified as anti-Jk^b. There is also a review of other cases of haemolytic disease of the newborn in which Kidd antibodies have been implicated.

Routine Compatibility Testing. Grove-Rasmussen, M. (1964), *Transfusion (Philad.)*, **4**, 200.

This paper records the results of a blood group antibody survey carried out with the co-operation of twenty blood grouping laboratories, and discusses the advantages to be gained from a carefully controlled screening test against a selected pool of red cells before crossmatching.

The Harmful Effects of Particles in Intravenous Fluids. Garvan, J. M. and Gunner, B. W. (1964), *Med. J. Aust.*, **ii**, 1.

This paper details the kinds of foreign particles that are often present in intravenous solutions, and their harmful effects on recipients. Suggestions are made for detection and improvement.

CHEMICAL PATHOLOGY

Separation of Serum Lactic Dehydrogenase Originating in Myocardial and Hepatic Tissue by Means of Heat Fractionation. Bell, R. L. (1963), *Amer. J. clin. Path.*, **40**, 216.

A method for serum lactic dehydrogenase estimation, using a buffer containing sodium pyrophosphate, sodium lactate and diphosphopyridine nucleotide is described. Experiments are performed to determine the normal range (incubation at 25°C.), the reproducibility of the method and the effect of varying the incubation time and temperature in cases

of myocardial infarct, infective hepatitis and normal patients. Using 130 blood donors, the normal range was found to be 55-147 units per ml. Ten cases of infective hepatitis had an LDH range of 100-356 units per ml. at 25°C. while there was a rapid and then slow decrease of LDH as the incubation time and temperature were increased. The LDH range of four cases of myocardial infarct was 247-543 units per ml. at 25°C. while, on increasing the temperature and incubation time there was a slow decrease and some activity after 60 minutes at 62°C. The author concludes that incubation at 60°C. for 60 minutes is best for differentiating LDH activity in the two pathological conditions; with a value in excess of 85 units per ml., under these conditions, being diagnostic of myocardial infarction. E.K.F.

Photometric Determination of Urinary Proteins. Saifer *et al.*, (1964), *Clin. Chem.*, **10**, 321.

A tryptophan method for detecting small amount of albumin and globulin at normal urine levels. An increase of globulin fraction is a sensitive indication of early renal disease. R.D.A.

A Rapid Semi-Automatic System of Chemical Analysis Using True Microspecimens. Walter, A. R. and Gerards, H. W. (1964), *Clin. Chem.*, **10**, 509.

20 μl amounts of blood or serum taken with a Gerarde micro pipette (B-D Unopette) and diluted in the plastic reservoir containing a suitable diluent. If a protein precipitant is used the reservoir may be centrifuged. All the supernatant can be decanted. Very versatile: sugar, protein, electrolytes and bilirubin quoted. Also useful for initial *Auto Analyzer* dilutions. R.D.A.

Direct Automated Determination of Glucose Oxidase Peroxidase System. Getchell, G. *et al.* (1964), *Clin. Chem.* **10**, 540.

A simple accurate automated version of a manual method by the same authors using glucose oxidase + o-dianisidine dye. High uric acid value caused a substantial decrease in values which could be minimised by using a greater plasma dilution. However, at a level of 15 mg. uric acid, recoveries were about 90%. R.D.A.

The Identification of Some Barbiturates by Thin Layer Chromatography. Shellard, E. J. *et al.* (1964), *Lab. Pract.* **13**, 516.

The application of gas liquid chromatography as a means of separating and identifying barbiturates is probably the method of choice, but if the apparatus is not available two dimentional thin-layer chromatography can be used to resolve limited mixtures of barbiturates. R.D.A.

Thin Layer Chromatography — Steroids: A Review. (1964), *Lab. Pract.* **13**, 306.

Silica gel + $CaSO_4$ run in n-butyl acetate located with Ceric SO_4 + H_2SO_4 tetrazolium salts + 2:4 dinitrophenol hyd. R.D.A.

Limitations in Clinical Use of a Screening Test for Proteins. Watson, D. (1964), *Clin. Chem.* **10**, 559.

Quantitative estimation in CSF is not feasible. The strip is relatively insensitive to gamma globulins, which could be greatly increased. Similarly Bence-Jones protein could be missed. R.D.A.

Venous Stasis and Forearm Exercise during Venipuncture as Sources of Error in Plasma Electrolyte Determinations. Broome, T. P. and Holt, J. M. (1964), *Canad. med. Ass. J.*, **90**, 1105.

In this study, blood samples were taken from the forearms of healthy adults to determine the extent of the error introduced into the determination of electrolyte levels by venous stasis and forearm exercise during venipuncture. Samples were taken from both arms of the volunteers, one arm being used as a control.

Venous stasis alone for two minutes introduced no significant errors, but continuous slow forearm exercise, simulating a difficult venipuncture,

resulted in clinically misleading elevation of the potassium and calcium levels.

Effect of Various Anti-coagulants on Carbon Dioxide — Combining Power of Blood. Zaroda, R. A. (1964), *Amer. J. clin. Path.,* **41,** 377.

After examining specimens of blood collected into different anti-coagulants, this author concludes that EDTA should be abandoned as an anticoagulant for determining carbonate levels in blood, as it yields lower results, which may be amplified in the presence of an excess of the anti-coagulant. Dried potassium oxalate is satisfactory, but the temperature at which it is dried must not exceed 180°C.

A Simple Chromatographic Screening Test for the Detection of Disorders of Amino Acid Metabolism. Efron, Mary L., Young D., Moser, H. W. and MacCready, R. A. (1964), *New Engl. J. Med.,* **270,** 1376.

This is a simple chromatographic method for the early detection of disorders associated with elevated blood amino acid concentrations. It uses dried blood on filter paper cards, and is suitable for mass-screening surveys.

CYTOLOGY

Exfoliative Cytology in the Diagnosis of Cancer of the Bronchus. Kuper, S.W.A., (1963), *J. clin. Path.,* **16,** 399.

This article describes a technique of examining sputum emulsified in 1% methlyene blue and gives results obtained by two independent observers. Also described is a technique to detect exfoliated cells in blood by removal of red cells with saponin and removal of polymorphonuclear cells by allowing them to phagocytose iron particles and exposing them to a strong magnet. After centrifugation and fixation the fluid is filtered through a *Millipore* filter then stained.

Tritiated thymidine was added to samples of serous effusions and blood, after incubation the specimens were treated as above and autoradiographs prepared, cells which take up the tritiated thymidine, mainly tumour cells, are shown by tiny black dots lying over the stained cells.

D.T.

HAEMATOLOGY

A Modified Method for Staining Neutrophil Alkaline Phosphatase and Normal Levels. *Wyllie,* R. G. (1964), *Med. J. Aust.,* **1,** 876.

This is a modification of the Gomori-Takamatsu method for the demonstration of alkaline phosphatase in blood films. The levels of alkaline phosphatase found in 100 normal adults fell between 0 and 30 units per 100 cells.

The Effect of Age on Normal Values of the Westergren Sedimentation Rate. Hilder, F. M. and Gunz, F. W. (1964), *J. clin Path.,* **17,** 292.

After carrying out Westergren sedimentation rates on bloods from 603 healthy blood donors, it was found that the normal values were higher than those generally accepted as normal. Also, the results showed an increase in normal values with age.

Thrombocytopenic Serum: an Artificial Factor VIII — Deficient Reagent. Davidson, E. and Tomlin, S. (1964), *J. clin. Path.,* **17,** 188.

Thrombocytopenic serum is a simple reagent derived from platelet-poor plasma. The high levels of factor V and zero levels of factor VIII in this reagent make it a reliable alternative to haemophilia A plasma in the quantitative and qualitative assessment of factor VIII in the thromboplastin generation test. [Author's summary] J.R.

A Useful Photometric Test for the Diagnosis of Von Willebrand's Disease. Vainer, H. and Caen, J. P. (1964), *J. clin. Path.,* **17,** 191.

When small amounts of adenosine diphosphate are added to citrated platelet-rich plasma, the consequent modification of platelets lead to a decrease in optical density. In Von Willebrand's disease the optical density at 610mμ increases instead of decreasing. This finding is used to study the effects of treatment on this disorder. [Author's summary] J.R.

HISTOPATHOLOGY

A Cresyl Fast Violet Stain for Bacteria and Fungi in Tissue. Puchtler, H. and Sweat, F. (1964) *Stain Tech.*, **39**, 1.

The technique employs Cresyl Fast Violet at pH3.7 which requires no differentiation and should be suitable for routine laboratory use.

B.G-J.

Staining Reticulin With Gold. Lynch, M. J. (1964), *Stain Tech.* **39**, 19.

Sections of formalin fixed tissue are exposed to 0.2% bromine water containing 0.01% KBr for 1 hour, rinsed and placed in an iodine solution containing KI for 5 mins. The sections are well washed in distilled water and immersed in 1% aqueous chloro-auric acid for 5 mins., rinsed again and the gold reduced in fresh 3% hydrogen peroxide or 2% oxalic acid. Reticulin fibres are dark brown to black or light blue.

D.T.

Basic Fuchsin and the Feulgen Reaction: Significance of the Dye for Cytophotometric Determination of Deoxyribonucleic Acid in Cell Nuclei. Lodin, Z., Muller, J., Pliny, J. and Hartman, J., (1963) *J. Histochem. Cytochem.*, **2**, 401.

A comparison of five brands of basic fuchsin as used in the Feulgen reaction for the quantitative estimation of the relative differences in the amount of deoxyribonucleic acid in cell nuclei. D.T.

MICROBIOLOGY

The Survival of *Haemophilus Influenzae* and Pneumococci in Specimens of Sputum Sent to the Laboratory by Post. May, J. R. and Delver, Doreen M. (1964), *J. clin. Path.*, **17**, 254.

The isolation rates of *H. influenzae* and pneumococci from fresh specimens of sputum are compared with those samples sent to the laboratory by post. The rate for both organisms from postal specimens is found to be approximately one half of that from fresh ones. The finding that postal specimens tend to be more acid than fresh ones does not seem to bear significantly on the survival of the organisms. H.C.W.S.

Colicine Production as an Epidemiological Marker of *Shigella sonnei*. Gillies. R. R. (1964), *J. Hyg. (Lond.)*, **62**, 1.

The paper describes modifications of the techniques used by previous workers and records the validity of the method as an epidemiological tool. Colicine typing of *Shigella sonnei* can apparently be performed more rapidly and economically by the method described. Type identification is possible within 36 hours of isolation without any loss of accuracy.

H.C.W.S.

A New Enrichment Medium for Salmonella. Banic, S. (1964), *J. Hyg. (Lond.)*, **62**, 25.

A new enrichment medium for the isolation of salmonella types from faeces is described. The inhibitory substances for coliform organisms and enterococci in this medium are magnesium chloride and sodium hydrogen selenite. In the reviewer's experience this medium has little if any advantage over existing, well tried broths, having similar inhibitory properties. Also it lacks any real control of Proteus strains. H.C.W.S.

Adsorption of Colicine. Mayr-Harting, Anna (1964), *J. Path. Bact.* **87**, 255.

The work described here deals with two aspects of the bacterial action of colicines, (1) the quantitative relation between effective colicine concentration and colicine diffusion and the number of sensitive bacteria, and (2) the nature of colicine receptors.

Considering the possible use of colicine typing of *Shigella sonnei* the results of this research may give us a better understanding of the problems involved. H.C.W.S.

The Routine Antibiotic Disc-Plate Sensitivity Tests. Barry, A. L. (1964),*Amer. J. med. Technol.,* **30,** 153.
Taken from a dissertation submitted to the Graduate School of the Ohio State University, this is the first of two articles dealing with sensitivity testing. Variation in size of inoculum is considered with excellent supporting bench work. Both articles may well produce a great deal of thought in relation to routine methods.

The Library

Librarian: J. Rees, Pathology Department, Medical School, Dunedin. List of Current Acquisitions:

Amer. J. med. Technol. Volume 30, No. 3. May-June 1964.
Contents: Evaluation of Staining Technics for Pathogenic Fungi; Use of a New Specimen Measuring Device for Hemoglobin. Total Cholesterol and Total Lipid Determinations; The Routine Antibiotic Disc-Plate Sensitivity Tests—1. Variations in the Size of the Inoculum; The Multiple Technic Concept—Its Application to Pulmonary Pathology; Immunofluorescence of Non-Cytopathic Tissue Culture Adapted Fixed Rabies; Film Strip Technic; Concentration of Small Volumes of Biological Fluids Using a Micro-Ultrafiltration Apparatus; Seasonal Variation of Anti-A Iso-Antibody in an 'O' Donor; The Use of MacConkey's Agar for the Differential Typing of *Mycobacterium fortuitum;* Blood—To Use or Not to Use; A Rapid Method for Serum Dilution in Anti-Streptolysin Titers; The Isolation of Coagulase Positive Enterococci from Clinical Material; Direct Counting of Platelets.
Aust. J. biol. Sci. Volume 17, No. 2. May 1964.
Canad. J. med. Technol. Volume 26, No. 3. June 1964.
Contents: A Simplified Dependable Procedure for the Laboratory Production and Storage of Streptolysin O; A Propos de la Nouvelle Classification des Enterobacteries; The Alkaline Phosphatase Activity of White Blood Cells; A Method for the Determination of Eight Tryptophan Metabolites in Biological Fluids.
Ann. Med. exp. Biol. Fenn. Volume 42, No. 1. 1964.
Selected Contents: A Human Rheumatoid-Like Substance Showing No Reactivity with Human Gamma-Globulin; Hereditary Serum Factors Gm(a), Gm(x) and Gm(b) in Finland; Antibody Formation in Pyelonephritis Patients after Vaccination against Salmonellosis; Studies in the Immune Response *in Vitro.*
Filter. Volume 36, No. 3. June 1964.
Contents: Laboratory Errors; New Methylene Blue Stain for Rapid Examination of Blood and Tissue Cells; Focus on the Future.
J. med. Lab. Technol. Volume 21, No. 2. April 1964.
Contents: A Simple Rapid Method for the Determination of Serum Calcium; Technical Considerations in the Cytochemical Demonstration of Neutrophil Alkaline Phosphatase; An Anamnestic Reaction Detected During Routine Crossmatching; A Comparison between Calcium Alginate and Cotton Wool in the Examination of Cattle for *Salmonellae;* A Modification of the Gitlow Screening Test for the Detection of Increased Excretion of 3-methoxy 4-hydroxy Mandelic Acid (V.M.A.) in Urine; Colour Reactions of the Trivalent Metal Halides as a Basis for the Estimation of Free and Ester Cholesterol in Serum; A Simple Test Tube Shaker and Incubator; A Note on 24 Hour Urinary Creatinine Estimation as an Index of Accuracy in 24 Hour Urine Collection; An Adaptation of the *Frigistor* Thermoelectric Stage Cooler for the Cutting of Paraffin Embedded Tissues.

Lab. Dig. Volume 27, No. 8. May-June 1964.
Contents: Simultaneous Determination of Glucose and Urea Nitrogen at a Rate of 60 per Hour; Interference in Lead Determinations by Dithizone; Questions and Answers in Microbiology; Biographical Sketch of Dr A. S. Wiener; Thyro-Binding Index (TBI); Abstracts.
Lab. Management. Volume 2, No. 4. July 1964.
Lab. World. Volume 15, Nos. 5, 6 and 7. May, June, July 1964.
Med. Surg. (Baroda). Volume 4, Nos. 5, 6, 7. May, June, July 1964.
Microbiologia (Buc.) Volume 9, No. 1. January-February 1964.
Contents: Digests and Abstracts of Papers Read at the Conference 'Aetiology and Epidemiology of Zoonoses.' (All in Rumanian.)
Offic. J. Amer. med. Technol. Volume 26, No. 2. May-June 1964.
Contents: Mechanism and Management of Hemorrhagic Diseases in Children; German Measles Infections; The Importance and Necessity of Continuing Education for the Medical Technologist; Mercuric Turbidity Test; Serum Beta-Lipoproteins; Bromsulfalein Test as an Aid to Liver Function; Wet Smear Cytology and Epithelial Cell Activity at the Time of Desquamation; Questions and Answers (For Problems in Preparing Solutions).
Rev. Viernes med. Volume 15, No. 1. April 1964.
S. Afr. J. med. Lab. Technol. Volume 10, No. 1. March 1964.
Contents: A Method for Determination of Copper in Urine.

Book Reviews

Steroid Determinations. P. K. Besch, Ph.D. and R. D. Barry, Ph.D., American Medical Technologists, 1964. 206 pages, 22 illustrations. Obtainable from the American Medical Technologists, 710 Higgins Road, Park Ridge, Illinois, U.S.A. at U.S. 5.25 dollars.

The copyright of this excellent book is held by American Medical Technologists, and to quote from the preface, 'This book is addressed to those persons who routinely carry out the major portion of the clinical determinations on biological specimens, the medical technologists. This book in no way attempts to serve as a reference text. . . .' In other words this is a practical bench book, written by practical people for the technologist who is going to carry out the practical procedure.

The introduction provides a description of the commoner steroids of physiological interest, classifying them and providing notes on their physiology and pharmacology. Under procedures, proved methods for the estimation of those steroids of current clinical interest are described in step by step detail. In the section on methods there are detailed discussions on paper, thin-layer and gas chromatography of steroids, whilst precise directions are given for the purification of solvents and materials. The chapter about research tools provides a guide to the uses of such tools as infra-red, visible and ultra-violet spectroscopes, mentions nuclear magnetic resonance spectrometers and gives an unusually good account of the theory of fluorescence. Other chapters give excellent descriptions of microchemical identification and reactions and radio-isotopes and radioactivity.

This small book has been carefully compiled to provide a working manual for the technologist in the difficult field of steroid analysis, and in this it has succeeded admirably. It is well referenced (up to 1963), and as well as its detailed step by step methods, it also indicates those techniques that will be finding use in the clinical laboratory in the next decade. Altogether the editors and authors can be congratulated on the conception and compilation of this work, which can be regarded as an

essential addition to the equipment of all laboratories where steroid analyses are undertaken. J.V.D.

Textbook of Microbiology. Eighteenth edition. Ed. W. Burroughs, Ph.D., J. W. Moulder, Ph. D. and R. M. Lewert, Sc.D. W. B. Saunders Co. Philadelphia, 1963. 1,155 pages. Local price £5 19s.

This book has been a standard text in microbiology for over 25 years, and has been extensively revised in the latest edition.

The layout is similar to that in most books on this subject, commencing with an historical introduction and working through laboratory methods, bacterial metabolism, genetics, taxonomy, immunity, individual organisms of medical importance, parasitology, mycology, *etc.*

The text is well up to date, with such topics as fluorescent antibody techniques, the newer penicillins and the anonymous mycobacteria all discussed.

Emphasis has been placed on the chapter by Doctor Moulder devoted to bacterial metabolism, and in seventy pages a bewildering array of metabolic pathways are covered. The parasitology section is skimped over in about sixty pages, covering a large number of parasites. *(Echinococcus granulosus,* for example, gets two columns and no diagrams.) One has the feeling that this subject is best left to the specialist book. Similarly with mycology, which is covered in fifty pages, including the *Actinomycetes.*

The general bacteriology section is well represented. There are some excellent photographs of colonial appearance and the various charts of biochemical reactions and so on are well prepared.

While this is an excellent text on the whole, and worthy of its place in a reference library, for the technologist who wishes to pay this much money Topley and Wilson is not much further away. R.T.K.

The closing feature of the 1964 Annual Conference at Wellington was the supper dance, held at the *Skyline,* Kelburn. Seen here are the President and Secretary with some of the official guests.

THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY (INC.)

20th Annual Conference

Held at Wellington Hospital on June 18 and 19, 1964.

The meeting opened at 9.15 a.m. with the introduction by the President, Mr H. G. Bloore, of the official guests.

Address of Welcome, Mr B. L. Dallard (Chairman of the Wellington Hospital Board).

In welcoming delegates to Wellington Hospital and to the capital city, Mr Dallard called attention, by mentioning the various conferences held recently at Wellington Hospital, to the fact that we are living in an age of specialisation.

Diagnosis and treatment, he said, was a matter of teamwork, calling for the integrated skills of specialists and technologists. With equipment becoming increasingly costly and its operation requiring an ever higher degree of skill, it seems that hospitals should give consideration to the centralisation of their resources. Parochial rivalries, resulting in wastage by the installation of expensive equipment where it would seldom be used, were to be avoided. To those who had the power to spend public funds in this way, Mr Dallard urged a pause for thought regarding the justification for and the potential use of expensive new apparatus.

Conference Address, Dr J. O. Mercer (Director of Pathology, Wellington Hospital)

Dr Mercer recalled that it was now exactly twenty years since he had been elected an Honorary Member of the Association of Bacteriologists, as our Institute was then called, and observed that there had been a considerable increase in numbers since that time.

Dr Mercer conveyed the warm good wishes of the New Zealand Society of Pathologists from their conference, held two weeks earlier in Wellington. The formation of the new Examination Board had been a mutually agreeable step, as had the finalisation of the new examination syllabuses. There had been, Dr Mercer said, a quickening of interest in formal training, and it was important to understand that progress means an improvement in overall standards all over the country, not merely local advances in some particular aspects.

Sketching the outline of a typical day's work in the Wellington Hospital laboratory of fifty years ago, Dr Mercer drew attention to the changes. A history of the Association would be well worth writing, he felt, for it was important to know what had gone before.

Opening Address, Dr G. Blake-Palmer (Director of the Hospitals Division, Department of Health)

Dr Blake-Palmer expressed his pleasure at being granted the opportunity, so early in his new office, to meet so broad a cross-section of medical technologists. He had met some of us in the course of his travels, particularly in smaller hospitals, and had been astonished at the ingenuity with which difficulties of space and facilities had been overcome. It was a tribute to the calibre of those concerned that the work was being carried out in laboratories, large and small, which were ill-adapted for the present day. New modern laboratories were at present being enjoyed at one or two favoured and not necessarily large hospitals, but there will quite soon be a spate of newer laboratories up and down the Dominion.

This was an interesting and challenging time, Dr Blake-Palmer declared, with the rapidly expanding pathology services depending upon the quality of skills and training which technologists as a group bring to their hospitals. There were encouraging signs of a healthy state regarding the

numbers and quality of those offering themselves for training to fit into an extremely diversified field of activity.

The new Medical Technologists Board had already had its first meeting and the new examination syllabus was soon to be printed. The natal form of this is, as with all such syllabuses, the product of many ideas and considerations. There may be reservations about what ought to have been included, but the achievement of an up-to-date, comprehensive syllabus represents a major step forward. In the light of experience, it will be reviewed from time to time, and this was as one would hope, for all good syllabuses evolve. Closely related was the question of the practical laboratory experience required for those entering the examinations. The problem of the laboratory with good working standards, but lacking the services of a whole-time pathologist, was one that may be possible to meet with an interchange of staff.

The Health Department will offer every encouragement and help towards the improvement of standards.

Presidential Address, Mr H. G. Bloore.

In his address, the President mentioned that the new Institute Rules and a Membership List had been printed; Associateship Diplomas completed; and that the Salaries Advisory Committee had met on December 14, but that there had, as yet, been no official announcement of their recommendations.

Regional voting for members of the Council had come into effect for the first time; and the first South Island seminar had been held at Timaru in April.

A sub committee had met representatives of the Nurses and Midwives concerning appeal machinery and this had been reported in the *Journal.*

The last meeting of the old Joint Committee and the first meeting of the new Medical Laboratory Technologists Board had taken place.

A sub committee had also met Messrs Wild and Mills concerning training. Mention was made of the proposed higher examination. The new syllabuses should be available in two or three months time and would be used as a basis for the examinations for the first time in 1966.

Roll Call

Name	Town
Aldridge, W.	Wellington
Allan, R. D.	Dunedin
Allen, Miss R. E.	Wellington
Bardsley, I.	Wellington
Bloore, H. G.	Blenheim
Bond, Miss H.	Wellington
Buchanan, Miss A.	New Plymouth
Buchanan, Miss M.	Rotorua
Buxton, I. R.	New Plymouth
Cameron, C. W.	Christchurch
Case, J.	Dunedin
Chambers, G. L.	Auckland
Coates, A. R.	Christchurch
Connelly, J. T.	Auckland
Cross, L. G.	Gisborne
Davies, J. A.	Whangarei
Davies, J. E.	Hamilton
Davies, Miss N.	Hamilton
Davis, G. F.	Auckland
Dodd, Miss D.	Christchurch
Donnell, M. McL.	Auckland
Dunlop, D. J.	Napier
Edgar, Miss J. M.	Dunedin
Fastier, L. B.	Upper Hutt
Fischman, A.	Auckland
Fitzgerald, D.	Timaru
Fletcher, E. K.	Dunedin
Ford, D. S.	Dunedin
Foster, H. E.	Tauramunui
Gardner, Miss G.	Wellington
George, G. R.	Rotorua
Glover, C. E.	Hamilton
Gratten, M. G.	Christchurch
Gray, Miss J.	Invercargill
Harding, Miss S.	Wellington
Hayes, Miss L.	Wellington
Hilder, F. M.	Christchurch
Hill, G. J.	Auckland
Hills, Miss M.	Wellington
Hitchcock, Miss D.	Nelson
Horner, J. E.	Ashburton
Howell, A. C.	Upper Hutt
Hudson, Miss M.	Christchurch
Jones, P. A.	Tauranga
Joyce, W.	Waipukurau
Kelman, Miss J.	Christchurch
Kennedy, R. T.	Auckland
King, I. C.	Auckland

Liardet, D.	Upper Hutt
Lumsden, Miss N.	Christchurch
Lun, Miss M.	Palmerston North
Lynch, M. J.	Wellington
Lyon, I.	Lower Hutt
McClure, Miss J.	Auckland
MacDiarmid, Miss H. J.	Hamilton
MacGibbon, N. A.	Wellington
McKenzie, R.	Masterton
McKinley, G.	Waipukurau
Maddocks, Miss P.	Wellington
Mattingley, Miss J.	Wellington
Meads, G. D. C.	New Plymouth
Meredith, J. G.	Auckland
Miller, T. E.	Auckland
Mitchell, D.	Dargaville
Mitcherson, B.	Hastings
Morgan, J. D. R.	Dunedin
Nixon, A. D.	Auckland
Norris, Miss D.	Wellington
Olive, H. T. G.	Wellington
Orbell, W. G.	Auckland
Parker, Mrs R.	Wellington
Paterson, Miss F.	Hamilton
Philip, D. J.	Auckland
Pittman, P. E.	Tauranga
Pybus, J.	Auckland
Rae, B. A.	Christchurch
Ranford, Miss H.	Wellington
Reeve, K. G.	Gisborne
Rhodes, Miss H.	Hamilton
Sadler, Mrs G.	Christchurch
Schwass, A. L.	Wellington
Shepherd, C. S.	Hamilton
Shooter, Miss G.	Wellington
Shott, H. C. W.	Dunedin
Sloan, W. J.	Auckland
Smail, R. W.	Invercargill
Smith, B. N.	Timaru
Smith, D. C.	Tauranga
Smith, F.	Napier
Stark, Miss G.	Wellington
Symonds, T. H.	Wellington
Tait, G.	Wellington
Taylor, L. R.	Oamaru
Taylor, Miss M.	Christchurch
Thomas, J. C.	Napier
Thompson, G. C.	Invercargill
Till, D. G.	Wellington
Toms, Miss V. M.	Wellington
Tucker, R.	Nelson
Wales, R.	Kawakawa
Walsh, J.	Auckland
Williams, A. H.	Palmerston North

Apologies were sustained from: Messrs Callaghan, Dixon, Jarman, Johnston, McCarthy, Rees and Ronald.

The meeting stood in silence in memory of Mrs Rundle and Mrs G. C. Thompson, who died during the year.

MINUTES OF THE TWENTIETH ANNUAL GENERAL MEETING

Moved:

That the minutes of the previous meeting be confirmed.

Allan/Fletcher Carried

Moved:

That the minutes of the previous meeting be signed as a true record.

Olive/McKinley Carried

Business arising

Mr Allan mentioned the remit on training.

Annual Report

In presenting the nineteenth annual report, the Secretary declared the total membership of the Institute as standing at 572 members, of which 519 were financial. There were 18 Honorary and 4 Life Members. New members elected during the year numbered 84. There has been one death and 4 resignations.

There were three meetings of the Council during the year.

The Secretary again thanked those in charge positions for the prompt return of staff questionnaires.

Business arising

Mr Allan mentioned the number of Council meetings and Mr Fischman spoke on the non-publication of papers presented at the last Conference.

Annual Statement of Account and Balance Sheet

The Treasurer presented the statement of account and balance sheet.

Moved:
That the Annual Report and the Annual Statement of Account and Balance Sheet be adopted.
Philip/Walsh Carried

Editor's Report

Moved:
That the Editor's report be adopted.
Case/Taylor Carried

Election of Officers

The following were elected to office for 1964/65:

President	Mr H. G. Bloore	unopposed
Vice-Presidents	Mr M. McL. Donnell	unopposed
	Miss J. Mattingley	unopposed
Secretary	Mr J. D. R. Morgan	unopposed
Treasurer	Mr D. J. Philip	unopposed
Council:		
Auckland Member	Mr R. T. Kennedy	unopposed
Wellington Member	Mr H. E. Hutchings	
Christchurch Member	Mr C. W. Cameron	unopposed
Dunedin Member	Mr E. K. Fletcher	

The President thanked Mr George and Miss Bond for their services to the Council and the Institute, and welcomed Messrs Cameron and Fletcher to the Council.

Moved:
That the rules of debate be observed.
Mitchell/Kennedy Carried

Notice of Motion previously circulated: (Auckland Branch)
Rule 8 (a) 3. The following shall be eligible for election by Council as Fellows:
'Those persons who at the time of the institution of the Fellowship Examination qualified for senior membership of the New Zealand Institute of Medical Laboratory Technology (Inc.) prior to August 30, 1949 and who since that date have been continuously engaged in the profession of medical laboratory technology.'
Miller/King

After considerable discussion, moved:
That the motion be put.
Shott/Case Carried

The motion was then put.
Defeated.

Remits previously circulated

Moved: (Auckland Branch)
That the Council of the New Zealand Institute of Medical Laboratory Technology (Inc.) makes a recommendation to the Examination Board to have the examination dates, both Intermediate and Final, brought into line with the normal academic year in that examinations should be conducted at the end of November each year.
Kennedy/Walsh Carried

Moved: (Dargaville)
That the N.Z.I.M.L.T. make vigorous representation to the Minister of Health to ensure that it becomes mandatory for Hospital Boards to ensure that their technologists and laboratory assistants (who are required to crossmatch blood for transfusion) have been given an extensive theoretical and practical course in Blood Bank Practice. In cases where the technician has not had the necessary specified training such a person is not to perform crossmatches unless under supervision of a qualified Blood Bank technician.
Mitchell/Davies Amended

After amendments (Olive/Wales and Lyon/Meads), the following motion was put:
That the N.Z.I.M.L.T. make vigorous representation to the Society of Pathologists seeking support to ensure that it becomes mandatory for Hospital Boards to ensure that their laboratory workers (who are required to crossmatch blood for transfusion) have been given an extensive theoretical and practical course in Blood Bank Practice. In cases where the laboratory worker has not had the necessary specified course of training, such a person is not to perform crossmatches unless under the supervision of a qualified laboratory worker.
Carried unanimously

Moved: (Dargaville)

That the N.Z.I.M.L.T. make vigorous representation to the Minister of Health to institute a compulsory standard technique, documentation and identification procedure for transfusion crossmatches in all N.Z. Hospitals.

Mitchell Lapsed (no seconder)

Moved: (Wellington Branch)

That the provision for Graduate Trainee be retained in the Regulations.

Toms/Bond Carried

Moved: (Dunedin Branch)

It be a resolution to Council from the A.G.M. that the following be eligible for election by Council as Fellows of the Institute.

Those members who since Certificate of Proficiency Qualification have been continuously engaged in Medical Laboratory practice, have been elected by Council as Associates and who at the time of the institution of the Fellowship examination have attained the level of Special, A or highest B grading or an equivalent standard where grading does not apply.

Withdrawn

Moved: (Dunedin Branch)

That steps be taken to ensure that all candidates in any one Health Department examination pertaining to Medical Laboratory Technology be notified simultaneously of their marks and/or success or failure.

Fletcher/Ford Carried

Moved: (Dunedin Branch

That the Council should make representations through the Institute's representatives on the Medical Technologists Board, to ensure that there is an early improvement in the standard of questions being asked in the examinations.

In particular, the practical examination that was in no sense practical (in this year's Intermediate) and the elementary nature of the practical questions in this year's Final Haematology paper are to be deplored.

Case/Shott Defeated

Moved: (Journal Committee)

That in order to safeguard the future of the Journal, all delegates intending to read a paper to an Annual Conference of the N.Z.I.M.L.T. (Inc.) shall henceforward be required to furnish the Conference Secretary, in advance of the date set for the Conference, with a typescript of the paper they intend reading, in a form suitable for publication. Further that the Conference Secretary shall then convey all such typescripts, as soon as possible after the Conference, to the Editor of the Journal for possible publication, either in full or in summary.

This remit was withdrawn and the following substituted (Case/Shott) with amendment (Fischman/Orbell).

That in order to safeguard the future of the Journal, this Conference urges delegates reading papers at all annual conferences of the N.Z.I.M.L.T. (Inc.) to prepare their papers in such a form as to be suitable for publication; and to submit an abstract as soon as possible after the conference to the Editor of the Journal for possible publication. Further that the Conference Secretary be urged to furnish the Editor with a complete list of papers read, together with the names of the authors, for inclusion in the reported proceedings of the Conference.

Case/Shott Carried

Moved: (Journal Committee)

That it be a resolution of this Conference that the Secretary of the Institute shall convey to the Editor of the Journal, the substance of all official communications from the Department of Health relating to final decisions regarding salaries, conditions of service, constitution of boards and committees et cetera; and that this shall be done as early as possible after the receipt of such communications, with the object of ensuring that the entire membership of the Institute becomes immediately aware of any changes.

Case/Morgan Carried

Moved: (Journal Committee)

That as an interim measure, until it becomes practicable to resume publication of the Journal four times annually, the months of publication of the Journal shall be readjusted so that the interval of time separating each issue becomes uniform. Commencing in 1965, the former April issue to be published in March, and the former October issue to be published in November; thereby establishing a regular four-monthly cycle of publication.

Case/Ford Carried

Moved:

That the ballot papers be destroyed.

Olive/McKinley Carried

Moved:

That the honoraria remain the same and be paid.

McKinley/Olive Carried

The meeting closed at 3.00 p.m. and was reconvened at 8.10 p.m.

The President reported on Mr Long's letter from the Hospital Board Association on liability and criminal charges.

Boards carry the risk for claims in civil proceedings and it would seem unlikely that the police would prosecute for criminal negligence. If a person should be required to make a statement in connection with the death of a patient, however, he would be wise to insist on the presence of his Pathologist or Medical Superintendent.

The Rex Aitken Memorial Prize:

No award this year, the £25 prize money being retained by Biological Laboratories Ltd., to enable the Award to be extended for a further year.

Junior Essay Prizes:

Essay Section: Adrienne Ramsay of Oamaru Hospital.

Accuracy and Quality Control in Clinical Chemistry

Technical Section: A. G. Wilson of the Medical School, Dunedin.

An Improved Method for the Estimation of Lipase in Serum, Using Phenyl Laurate as a Substrate.

Congratulations were extended to the winners and it was directed that cheques for £5 5s 0d be sent to them both.

Moved:

That the Nurses be supported in their approaches to the Salaries Advisory Committee to determine where and why submissions are turned down; this support to be given as Council sees fit.

King/Toms Carried

It was resolved that no application be made to the S.A.C. calling for the observance of January 2 as a statutory holiday.

Moved:

That Conference recommends to the Medical Laboratory Technologists Board that the Intermediate Examination be given a status in its own right.

Hilder/Shepherd Defeated

The meeting carried a vote of thanks to the Conference Committee (Messrs Aldridge, Beattie, Olive and Symonds, Miss Toms, Mrs Godkin and Mrs Stewart) with acclamation.

Offers were received from Tauranga and Nelson for the venue of the next conference.

On being put to the vote, Tauranga was chosen.

Moved:

That the auditor be reappointed.

King/Chambers Carried

Moved:

That the form of the Associateship Certificate approved in December 1963 be abandoned and the Council be instructed to produce a Certificate along the lines of the certificate shown to the meeting. The personal details to be written on it in script.

Walsh/Miller Carried

Moved:

That the honoraria be: Secretary £10 10s 0d; Editor £10 10s 0d; Treasurer £7 7s 0d; Auditor £5 5s 0d; Scrutineer £2 2s 0d.

Fletcher/Ford Carried

Moved:

That the earlier motion regarding honoraria be rescinded.

McKinley/Aldridge Carried

The meeting closed at 9.55 p.m.

Papers Read at the 1964 Annual Conference

BACTERIOLOGY FORUM (Chairman: Dr J. D. Manning)

Identification of Gram-negative Bacilli in Urine Mr J. G. Meredith

Screening of Urines for Culture Using Bacterial Counts Mr J. G. Meredith

A Case of Renal Disease with a Telescoped Urine Deposit Mr T. E. Miller
Medical Technology in South Vietnam Mr R. McKenzie

BIOCHEMISTRY FORUM (Chairman: Dr F. Desmond)

Thin Layer Chromatography Miss J. Mattingley
Comparison of Urastrat and Autoanalyzer Ureas Mr J. G. Meredith
Problem of Screening for Phaeochromocytoma Mr E. K. Fletcher
Estimation of Protein Bound Iodine by Chloric Acid Digestion Mr J. Pybus

HAEMATOLOGY FORUM (Chairman: Dr A. J. B. Erenstrom)

A Review of the Pelger-Huet Cell Anomaly Mr B. A. Rae
Pyruvate Kinase Deficiency Anaemia—Report of Laboratory Findings in One Case Mr A. D. Nixon
Detection and Identification of Atypical Antibodies Mr D. S. Ford

IMMUNOLOGY FORUM (Chairman: Dr L. B. Fastier)

The Clinical Application of Haemagglutination Tests Mr H. C. W. Shott
The Detection of Infection Process by Fluorescent Microscopy Dr L. B. Fastier
Notes on a New Technique for Treponemal Antibodies and Polysaccharide Antigens Mr A. Fischman

Trade Displays at the 1964 Conference

Abbott Laboratories Ltd.
Biological Laboratories Ltd.
Burroughs Wellcome and Co. (N.Z.) Ltd.
Dental and Medical Supply Co. Ltd.
Dentists Supplies Ltd.
Dominion Dental Supplies Ltd.
Ethicals Ltd.
H. T. Frost Ltd.
Manufacturing Laboratory Supplies Ltd.
National Dairy Association of New Zealand Ltd.
Edwin A. Piper Ltd. *(Oxoid)*
Potter & Birks (N.Z.) Ltd.
Sonotone Ltd. *(Zeiss)*.
Townson & Mercer Ltd.
William R. Warner & Co. Ltd.
Watson Victor Ltd.
Geo. W. Wilton & Co. Ltd.

The Conference Committee were indebted to the following firms for gifts:—

E. C. Lackland & Co. Ltd—Key rings for male visitors.
W. & R. Smallbone Ltd—Vincent's Powders for hotel guests.
W. D. & H. O. Wills Ltd—Cigarettes for the cocktail party.

The Twenty-first Annual Conference N.Z.I.M.L.T. (Inc.)

will be held at

TAURANGA

on

JULY 1 and 2, 1965

Council Notes

A meeting of the Council took place at Wellington Hospital on June 17, 1964. Present were: Mr H. G. Bloore (in the Chair); Misses H. M. Bond and J. Mattingley; and Messrs J. Case, M. McL. Donnell, G. R. George, H. E. Hutchings, R. T. Kennedy, J. D. R. Morgan and D. J. Philip.

Technical Certification

In pursuit of constructive ideas for improvements in training, examination and certification of medical laboratory technologists, the Council was addressed by Mr Wild, Chairman of the Technicians' Certification Authority.

Mr Wild explained that the Authority had come into being as a result of the Technicians Certification Act (1958), and existed for the purpose of approving courses of training, conducting examinations and issuing certificates to technicians generally. The fields covered at present include science, engineering and building, and it seems possible that medical laboratory technology could be included if this were considered desirable.

The discussion lasted for some time and Mr Wild answered many questions, but no decision was reached as it seems that there remains some doubt whether the technical institutes in New Zealand will be able to provide the highly specialised courses necessary. The matter is not, in any case, one that can be settled by the Institute alone. The decision would rest with the Department of Health, which would, presumably, be guided by the recommendations of the Medical Technologists Board.

Medical Technologists Board

The President reported on the first meeting of the new Board on June 5. It is not clear, at present, if it is the intention of the Department of Health that the Board will be an advisory committee whose deliberations will be secret, or whether the various members should be regarded as representing the organisations that nominated them, with the right to report back to those organisations. Mr Bloore said that it had been the hope of the nominees of both the N.Z.I.M.L.T. and the Society of Pathologists that they will be permitted to discuss the meetings of the Board with their respective bodies.

A Special Examination will be held in October for the five candidates who obtained partial passes in the 1964 Finals. The written papers will be undertaken on October 5 and the practical papers and oral examination will be held at the National Health Institute, Wellington, on October 22 and 23.

Annual Trophy

The Council accepted, with grateful appreciation, an offer from Watson Victor Ltd. to award a trophy for presentation to the top student in the Final Examination each year, which it has been suggested should be held at the winner's laboratory for the twelve-month period until the next examination. The precise form of the trophy has not been decided.

Legal Responsibility

The question of the responsibility in law of a technologist who may be implicated, through some error of carelessness or omission, in the death of a patient, has not been fully resolved. Correspondence with the Hospital Boards Association had proved unsatisfactory inasmuch as the Association has failed to appreciate that the Institute's main concern is with the measure of legal assistance and protection individual hospital boards would offer a technologist in the event of criminal proceedings for negligence. The matter of civil litigation for damages is a different matter entirely, and one that would seem to be covered by the technologist's responsibility

to his medical superior, who would generally have adequate personal insurance against such an eventuality.

Public Service Association Shopping Facilities

An application was made some years ago for N.Z.I.M.L.T. members to be considered eligible for the shopping discount privileges enjoyed by members of the P.S.A. This application was refused, but it is understood that, in the meantime, a few individual members have applied for and been granted these facilities. A further formal approach will be made by the Secretary.

Applications and Resignations

In addition to the list published in the July *Journal,* the following applications and resignations were approved:—

New Member

Hickey, Miss P. .... Auckland

Associates

Aldridge, W. .... Wellington   Olive, H. T. G. .... Wellington

Resignations

Hodgetts, Mrs J. .... Wellington   Lane, Mrs D. P. .... Nelson

Ex-Members of the Joint Committee

Entered in the official record were the thanks and appreciation of the Council for the work of Messrs H. T. G. Olive and D. Whillans as members of the Joint Committee, now disbanded and replaced by the Medical Technologists Board.

Branch Reports

DUNEDIN

(Secretary: E. K. Fletcher, Pathology Department, Medical School.)

The 1964 Annual Conference in Wellington was attended by twelve of our members, three of whom presented papers, while all contributed to the carrying of the majority of our remits. Our congratulations and thanks to the Conference Secretary and Wellington members for a most successful conference.

A 'work' evening was held in July, when we were visited by members of the New Zealand Institute of Science Technicians. Thirty visitors showed great interest in the demonstrations and displays. Displays were judged for their presentation and informative value, and the trainee organisers of two (electrophoresis and red cell fragility) were awarded cash prizes.

Our conference delegates reported back at the August meeting and afterwards we visited the Surgery Department where Mr Borrie, Thoracic Surgeon, demonstrated the heart-lung bypass machine. Saline was used in place of blood in a working demonstration. Nearby, post-operative four legged patients were nonchalantly eating hay.

At our September meeting we intend to hold a discussion on the training and education of medical laboratory technologists—after which there will be an address by a member of the staff of the Physiology Department. On September 30th, we hold our Annual General meeting. The year's activities will close with a social function in December.

E.K.F.

WAIKATO — BAY OF PLENTY GROUP

Forty-two members, representing six laboratories, attended an afternoon meeting at Tauranga Hospital on Saturday, August 15. The following papers were read and discussed, after which there was an inspection of the new laboratory, a tour of the new hospital and afternoon tea.

Contributions were:—

Training of Laboratory Technologists in Australia:
Miss M. Main (Private laboratory, Hamilton).
Desoxyribonucleic Acid: Miss J. Prior (Waikato Hospital)
A Case of Pasteurella septicum *Infection:*
Mr D. Henry (Private laboratory, Tauranga).
The Use of Plastics in the Laboratory:
Miss L. Eccershall (Waikato Hospital).
Newer Methods of TB Isolation: Mr M. Hampson (Rotorua Hospital).
A Discussion on Serum Calcium Estimation:
Chairman—Mr B. Barry (Private laboratory, Hamilton).
A Case of Erythroleukaemia: Mr D. Quinnell (Tauranga Hospital).
Demonstration of Cryostat Section Cutting:
Mr R. Reilly (Tauranga Hospital).

The next meeting is to be held at Hamilton in November.

D.C.S.

Auckland Hospital Board School of Medical Laboratory Technology

1964 Prize-Giving Ceremony

The Annual Prize Giving Ceremony at the Auckland Hospital Board School of Medical Laboratory Technology was held on Thursday evening, July 23, 1964, in the Medical Centre, Auckland Hospital.

Those attending were the Medical Superintendent-in-Chief, Dr W. E. Henley, the Director of Laboratory Services, Dr S. E. Williams; the Chairman of the Hospital Board, Mr T. H. C. Caughey; and qualified and trained medical and technical staff from private and hospital laboratories.

Over 100 people were present when the meeting was formally opened by Dr S. E. Williams.

This was followed by brief summaries of original work carried out during 1963 by fourth-year trainees. The top eight trainees, whose work was selected for presentation were:—

Mr M. Ford: *The Estimation of 3-Methoxy 4-Hydroxy Mandelic Acid as an Aid to the Diagnosis of Phaeochromocytoma.*

Miss A. James: *Fever, Splenomegaly and Abnormal Lymphocytes: A Syndrome Observed after Cardiac Surgery Utilising a Pump Oxygenator.*

Mr N. Davy: *An Investigation into the Efficiency of Aqueous Benzalkonium Chloride as a Disinfectant.*

Mr W. Beggs: *The Detection of Phenylketonuria in New Zealand.*

Mr A. James: *A Simple Micro-method for the Estimation of the Specific Gravity of Urine by a Falling Drop Method.*

Miss S. Holland, Mr R. Wong Too: 1. *An Investigation and Comparison of Routine Tests for Staphylococcal Pathogenicity.*
2. *The Use of Mercuric Chloride in Identifying Multiple Antibiotic-Resistant Staphylococci.*

Miss R. McBride: *The Lathe and Ruthven Method for Estimating the Conjugated and Unconjugated Bilirubin in Comparison with an Adaption of the Powell Method.*

Dr W. E. Henley then addressed the meeting, making special reference to rapid development of laboratory diagnostic procedures, and reminding all of the fact that those working in the various fields of medicine should never lose sight of the well-being of the patients when striving for better standards.

The prizes, consisting of books donated by George W. Wilton and Co. Ltd., Dental and Medical Supply Co. Ltd., E. C. Lackland and Co. Ltd., and Watson Victor Ltd.; and certificates were then presented by Mr T. H. C. Caughey.

First Year Trainee: Mr N. Yeates.
Second Year Trainee: Mr N. Anderson.
Third Year Trainee: Miss N. Turley.
Fourth Year Thesis Prize: Mr M. Ford.
Fifth Year Trainee: Mr J. McLachlan.

The Board's Certificate for technologists qualifying from the Auckland School was awarded to Mr J. McLachlan.

In addition, certificates were presented to qualifying technical assistants in Intravenous Solutions Technique, Virology Technique, Medical Cytology, Paediatric Biochemistry, Histological Technique and Blood Bank Practice.

The formal part of the evening concluded with a reply by Mr D. Whillans, Principal Medical Laboratory Technologist, during which he particularly thanked the Auckland Hospital Board for their help with various aspects of training, and Dr S. E. Williams for his whole-hearted support in the establishment of the School. R.T.K.

Vacancies

MEDICAL LABORATORY TECHNOLOGIST

A PROGRESSIVE commercial laboratory situated on the North Shore, Auckland, requires a recently qualified male medical laboratory technologist for their control and development section. The successful applicant will be given every opportunity to further his career in the commercial aspects of laboratory work and a salary commensurate with his ability and experience will be paid.

All applications will be treated in the strictest confidence.

Apply in the first instant, in writing, stating age, marital status and qualifications to:

The Manager,
BIOLOGICAL LABORATORIES LTD.,
Private Bag,
Northcote,
Auckland.

BACTERIOLOGIST
AUCKLAND REGIONAL AUTHORITY DRAINAGE DIVISION

Applications are invited for the position of works bacteriologist at the Manukau Sewage Purification works at Mangere.

Applicants should be experienced in the field of general bacteriology and hold appropriate qualifications.

The position offers interesting work in a modern laboratory. The salary will be in the range £1,059-£1,336 per annum depending on qualifications and experience.

Conditions of appointment are available on request.

C. C. BLOW,
Secretary.

Drainage Division,
P.O. Box 208.
Auckland, C.1.

LABORATORY TECHNOLOGIST
MEDICAL UNIT, WELLINGTON HOSPITAL

A Laboratory Technologist is required to take part in a 2-year research project concerning electrolytes and acid base.

Salary will be in accordance with the Hospital Employment (Laboratory Workers) Regulations, the commencing rate to be determined in accordance with qualifications and experience.

Enquiries should be directed to Dr K. A. K. North, Acting Director, Medical Unit, Wellington Hospital.

Letters To The Editor

EDITORIAL VERBIAGE

Sir:

Personal awareness dictates I must take you to task over your editorial in the July issue of the *Journal* and to a much lesser extent over the enclosure appearing in the centre pages. If my letter incites further discussion, for or against, I shall feel extremely pleased, for an interest in our profession is essential and can sometimes best be served by controversial issues. Admittedly you have some worthwhile points in your editorial, but they are buried beneath a pile of irrelevant verbiage.

You ask, 'Is an honest mistake, made through ignorance, a crime?' I reply that there is no place in any blood bank for ignorance, and it is the duty of the pathologists and senior technologists to ensure that this situation does not arise. YES, ignorance in blood bank *is* criminal.

You also state, 'It is worthwhile bearing in mind that if a patient dies, either directly or indirectly as the result of an error of omission on the part of a medical laboratory technologist, criminal proceedings may follow.' To assist your readers—Heaven help them—I offer you the following to help calm the waters you have ruffled.

SECTION 86 of the HOSPITALS ACT 1957.

86. Liability of Board for negligence:

Where damage is suffered by any person as a result of any wilful or negligent act of any medical practitioner, dentist, matron, nurse, midwife, attendant or other person employed or engaged (whether in an honorary capacity or otherwise) by any Board, and acting in the course of his or her employment or engagement, an action in respect of the damage shall be against the Board by or on behalf of the person suffering the damage, and in any such case the Board shall be liable in the same manner and to the same extent as if the damage had been caused by an act or omission of a servant of the Board acting in the course of his employment.

SECTIONS 155 and 157 of the CRIMES ACT 1961.

155. Duty of persons doing dangerous acts:

Everyone who undertakes (except in the case of necessity) to administer surgical or medical treatment, or to do any other lawful act the doing of which is or may be dangerous to life, is under a legal duty to have and to use reasonable knowledge, skill and care in doing any such act, and is criminally responsible for the consequences of omitting without lawful excuse to discharge that duty.

157. Duty to avoid omissions dangerous to life.

Everyone who undertakes to do any act the omission to do which is or may be dangerous to life is under a legal duty to do that act, and is criminally responsible for the consequences of omitting without lawful excuse to discharge that duty.

I should be grateful if you could explain to me the authority behind the procedure printed in the centre pages which you say, 'It is hoped that it will be torn out and placed in a prominent position on the wall of every laboratory.' I suspect that these are personal views, but nowhere on the pages referred to is there any reference to this. Bearing in mind the fact that there is a selected panel, suitably qualified, already formulating such a standard technique—this was why a remit was reworded at the last Annual General Meeting—I cannot help feeling there may be some confusion in that some technologists could assume that this was the result of the panel's deliberations. There appears to be no direct acceptance of responsibility on anyone's shoulders as to the authority behind the procedures appearing under the heading *Towards Greater Safety in Blood Transfusion.* Would you please rectify this omission.

Regarding your recommendation to label the specimen container BEFORE withdrawing the blood sample I can only hope that your centre is the only one following this practice. You cannot identify any container as truly containing any sample until the sample is safely inside the said container. Mistakes could occur using both techniques granted, but I feel there is less chance if the labelling is done as the second step and not the first.

I. C. KING,

July 28, 1964.

[1. We heard at the last Annual Conference that Sections 155 and

157 of the Crimes Act are seldom likely to be invoked, but they are on the Statute Book and the threat of prosecution exists. This being so, I still consider that the Hospital Board that protects its employees from the possibility of making a damaging admission to a policeman, is undeserving of the public censure inflicted by a coroner on the Board in the recent case.

As to whether or not the Crimes Act ought to contain those particular sections, I think it is not irrelevant to observe that 'The Law is an ass' if it thinks it can deter people from making mistakes by threatening punishment.

2. If 'Ignorance in blood bank is criminal,' then it is a great pity that candidates for the Certificate of Proficiency Examination are not expected to have a very much more detailed knowledge of blood group serology. As far as I am concerned, this is a sore point.

3. My use of the passive voice in the sentence, 'It is hoped that it will be torn out and placed in a prominent position on the wall of every laboratory' in no way changes the obvious fact that since the editorial was signed with my initials it was I who was hoping it.

It is implicit in the *absence* of any authority for the procedures printed on the centre pages of the July *Journal,* that these are suggestions from a personal viewpoint, and you can take it that *everything* printed within these pages represents an individual opinion unless specifically stated to be otherwise. I cannot take seriously this correspondent's suggestion that some technologists may have assumed that the suggested procedures are the result of a selected panel's deliberations, for only the feeble-minded would expect the Voice of Authority to speak through the medium of our journal.

Since the publication of the July *Journal,* all Hospital Board Secretaries and the Superintendents of all Public Hospitals will have received a copy of Department of Health Circular Letter No. Hosp. 1964/84, dated August 20, entitled: *Criteria for Acceptance of a Voluntary Blood Donor, Requirements in Patient Identification and Minimum Standards of Technical Handling of the Blood.* This presumably contains the recommendations of the suitably qualified panel to which Mr King alludes. I am pleased to say that there appears to be no conflict between my suggestions and the Department's recommendations; although there are some instances in which I have been more explicit. It would be possible to quote several authorities for the conviction that a saline and anti-globulin crossmatch is inadequate, but the official recommendations do suggest this as a *minimum* requirement, so we can probably add as many additional tests as our consciences may dictate. This is an interesting document because besides outlining the requirements for the acceptance of a donor, information necessary on requisition forms and labels for blood grouping tests, suggestions about the technical handling of blood and other kindred matters, it also details the proposed new uniform system of labelling donor blood which cannot come into operation too soon for safety.

4. I do not anticipate difficulty in justifying my assertion that the safest time at which to label a specimen container is immediately before the sample is put into it; but I must say, first, that the most reliable safety regulations are those drawn up on the basis of local experience. Anyone subject to an official decree that he must label specimens immediately *after* collection must, of course, do exactly that. If he adheres, strictly, to the letter of his instructions, he can scarcely go wrong. However, my point is that the method I suggested renders the adherence to that letter simpler and more certain.

To serve its purpose properly, the label on a sample for blood grouping must carry the patient's *full name*, his date of birth and his hospital or unit number. This information stands the greatest chance of being faithfully and completely recorded if this is done immediately following the interrogation that establishes the patient's identity. The details are fresh in the mind and there are no distractions. Leave the labelling until afterwards and there are numerous reasons why it may be incorrectly done. There is a dirty syringe to dispose of; if it is a non-disposable one it will have to be rinsed; you have to make sure that the patient is holding the swab against the venipuncture site (sometimes you have to hold it there yourself); you may have to mix a part of the specimen with an anticoagulant; there are the inane observations a patient will often make when his tension is released by the discovery that the operation was less painful than he had feared, not to mention the playful badinage of his fellow-patients; and, already, if you are busy, your mind will be on the next patient you have to bleed. Under these conditions it is not surprising if the sample gets labelled incompletely; is labelled with the name of the next patient, or with the doctor's name; or not labelled at all. These errors lead to identification by assumption or by elimination, which must be avoided at all costs.

In all honesty, I fail to see what can go wrong if the labelling is done before venipuncture. If your routine practice is to go to the bedside, identify the patient, take out and label a bottle, then immediately withdraw the specimen and discharge it into that bottle, there is no way for you to make a mistake. At no time is the sample of blood anonymous. When it is in the syringe it is connected to the patient through the needle you have in his vein; immediately you withdraw the needle it is connected to a bottle with his name on it, through the stream of blood flowing through the nozzle of the syringe.—J.C.]

EDUCATION FOR MEDICAL TECHNOLOGISTS

Sir,

There is a tendency to assume that the circumstances we find ourselves in are unique. The duties medical technologists undertake in New Zealand are substantially the same as those performed elsewhere. The exigencies of specialisation are upon us, the need for staffing outlying areas occurs here as elsewhere, and the paradox of using trainees to perform the routine work disorganises laboratories in all the far flung corners of the world.

The changing needs of technology and methods of education have at length repercussed into medical laboratory training overseas. The inefficient method of apprenticeship, which stems from the craft societies of the Middle Ages, is being replaced by systematic part-time education.

As you may know, education for medical laboratory technologists was currently discussed in the *Lancet*, February to April 1964, and in the British I.M.L.T. *Gazette*, August 1964. Many a familiar echo is heard. The shortage of trained personnel was blamed on inadequate or old fashioned teaching 'students should learn more basic science and this is best taught by qualified teachers in technical colleges' and, again, instruction in the basic sciences from technical colleges was necessary if career prospects were to be opened up so as to overlap with science graduates 'an essential step in the realisation of the potentialities of the more gifted technicians' schools of medical laboratory technology and two-tier systems were also suggested but not very convincingly. Two members of the British Medical Laboratory Technicians Registration

Board, Dr Joan Stokes and Mr W. Davey, Principal of the Portsmouth College of Technology, have contributed articles on teaching in the British I.M.L.T. *Gazette*. Dr Stokes remarks on the advantages of *block* release as against *day* release in that a series of block releases can be staggered, and the same ground covered for different groups of students so that the laboratory is not deprived of staff; and also on the difficulties confronting students working in rural districts.

Mr Davey is very much aware of the need to plan for the future, and of preventing Medical Laboratory Technology from becoming a depressed occupation. To do this it is necessary, he states, to see that prospects, working conditions, education, training facilities and rewards (in terms of status, job satisfaction and salary) are equal to the opportunities offered in other professions. He remarks: 'At present the old apprentice training by "standing next to Nellie" has been replaced by more formal courses of instruction.'

In the Dominion we are still casting around for a system of education, and it was depressing to find at the last conference that very little constructive thought had been given to the topic, while the pros and cons of curlicues on the Diploma were discussed with verve and animation. This was akin to discussing the colour of paint to be applied to the cart which we propose to place before the horse.

To my mind, we have an admirable opportunity to systematize our training by making use of the syllabus constructed by the Technicians Certification Authority in basic science subjects. It is true that many trainees have already received a grounding at school. In my experience their knowledge is invariably sketchy, and I have yet to meet a trainee sufficiently conversant with the fundamental chemical concept of equivalence to apply it practically. Furthermore, the gap between school science and, say, the genetical background of Blood Group Serology, or the simple electronics of a spectro-photometer, is not one that can be bridged by a few casual words at the bench. Indeed, it requires the training and experience of the professional teacher to impart the knowledge adequately and provide a proper grounding in the correct sequence. It was, in my opinion, unfortunate that such a pessimistic view of prospects for technological education should have been presented at the Conference. I would have thought that at this stage support and encouragement was in order for a project which might prove a boon and a blessing to us.

It is true that some of the subjects included in the Technicians Certification Authority Handbook await suitable staff and sufficient demand to be implemented. It is also true that training schemes to provide the staff are under way. Many of the subjects offered in the later years of the course would round out our education admirably. I refer to Genetics, Physiology and Biochemistry. I see no difficulty, other than administrative ones, in suitable combinations of their courses with, in parallel, a subsequent teaching of medical laboratory specialities by medical laboratory technologists of suitable talent, adequately briefed, in or out of technical institutions.

I do not believe there is a simple solution to the problem of staffing and training in outlying laboratories, nor of reconciling the need to provide staff for routine work and sanctioning day or block release. I can, however, think of several workable solutions to both these problems.

R. D. ALLAN.

28th August, 1964.

Directions for Contributors

In the interests of uniformity, the following directions should be followed by all contributors to the Journal.

Manuscripts should be typewritten on one side only, of good quality quarto sized paper, be double spaced and with a 1½in margin. They should bear the author's name (male authors give initials, female authors one given name), address, and (if this is different), the address of the laboratory where the work was carried out. The manuscript should take the following form: Introduction; materials and methods; results; discussion and conclusion; summary; acknowledgments; references. Carbon copies are not acceptable. Nothing should be underlined unless it is necessary that it be printed in italics.

ILLUSTRATIONS. Graphs and diagrams are termed 'figures' and should be numbered in the order of their appearance in the text. Figures should be drawn in Indian ink on stout white paper larger than required for the text. Legends to the figures should be typed separately and attached. Illustrations, particularly half tone blocks, should be sparingly used. Half tone blocks are referred to as 'plates' and these again are numbered in sequence and the captions are typed and attached. Elaborate tables should be kept to a minimum but any necessary tables should be typed on separate sheets of paper and numbered in roman numerals.

NOMENCLATURE. Scientific names of micro-organisms should conform with the system adopted in the latest edition of *Bergey's Manual of Determinative Bacteriology* and underlined to indicate that they are to be printed in italics. Collective names for groups of bacteria such as staphylococci, salmonellae, etc., should not be underlined. Abbreviations such as C.S.F. for cerebro-spinal fluid, are only permissible if their meaning is clearly indicated when first introduced. Conventional abbreviations such as ml. for millilitre and cmm. for cubic millimetre are acceptable without explanation. Names of chemical substances should conform to current chemical practice and care should be taken to see that chemical formulae are correct.

REFERENCES: Only papers closely related to the author's work should be quoted. Contributors should study this issue of the Journal for examples of the preferred method of making reference. All references are brought together at the end in alphabetical order and numbered. In the list, references should include (1) Surname followed by the initials of the author(s), (2) Year of publication in brackets (3) Abbreviated title of the periodical according to *World List of Scientific Periodicals* or to *World Medical Periodicals* (underlined), (4) Volume, (5) Page numbers. If there are three or more authors the words *et al* may be added to the name of the first author in the text, but the names of all co-authors must be given in the list. References to books should include (1) Author(s) or Editor(s), (2) Year of publication in brackets, (3) Title (underlined), (4) Edition, (5) Page number referred to, (6) Name of Publisher and Place of Publication.

REPRINTS. Authors may receive a minimum of 50 reprints at cost price. These should be ordered when returning corrected galley proofs.

PROOFS. Whenever time permits, authors will have the opportunity to correct galley proofs before publication. No major alterations will be permitted unless the author is prepared to stand the cost, and proofs must be returned within three days of receipt.

Index to Volume 18

Abstracts - 33, 77, 133
Annual Conference, 1964 - 140
Annual General Meeting Minutes - 142
Accuracy and Quality Control in Clinical Chemistry - 125
Auckland Prizegiving - 149
Associates Elected - 94, 148
Book Reviews - 40, 138
Branch Reports - 43, 88, 148
Brown, T. E. - 100
Canadian Technologists - 7
Comparison of Methods for the Estimation of E.S.R., A. - 25
Council Notes - 42, 147
Curtis, P. H. - 69
Dawkins, B. F. - 55, 115
Determination of Serum Calcium Using the E.E.L. Flame Photometer, The - 52
Editorials - 2, 50, 98
Examination Papers - 38, 83
Felmingham, C. E. - 66
Fletcher, E. K. - 9
Grey, M. Jeannette - 19
Improved Colorimetric Method for the Estimation of Lipase in Serum, An - 119
Irradiation Plants for Medical Sterilization - 72
Lactic Dehydrogenase and the Application of its Estimation in the Routine Clinical Laboratory - 100
Letters to the Editor - 32, 92, 151
Library List - 45, 95, 137
Lithium Sequestrene as an Anticoagulant - 61
McLean, B. - 52
Macroglobulinaemia — A New Case - 19
Medical Laboratory Technologists Board - 51
Micro-Drop Technique for the Titration of Anti-Streptolysin O in Human Serum, A - 115
Montgomerie, Jillian E. - 25
New Members - 43, 93, 148
One Day Seminar at Timaru - 89
Problem of the Collection of the Midstream Urine, The - 69
Question of Responsibility, A - 50
Ramsay, Adrienne - 125
Sentance, Joanna - 61
Simple Apparatus for the Estimation of Alveolar pCO_2, A - 66
Status and Training - 2
Suggestion for Future Training of Medical Laboratory Technologists, A - 89
Survey of Red Cell Indicator Systems for the Staphylococcal Anti-Alpha Haemolysin Test, A - 55
Symonds, I. H. - 52
Total Serum Cholesterol Estimation - 9
Thermo-Electric Cooling - 130
Training and Examinations - 98
Wilson, A. G. - 119

We illustrate our WB. 400 Water Bath 14" long x 12" wide x 6½" deep with two stainless steel racks each accepting twenty-four 5" x ⅝" tubes.

This well designed bath features the latest developments in temperature control and engineering techniques, and is now manufactured in New Zealand for your benefit.

CONTHERM
UNIVERSAL
WATER BATHS

* Uniform, accurate temperature control to 100°C.
* Temperature fluctuation 0.3°C. at 37°C.
* Graduated thermoswitch dial, indicating pilot lights.
* Polished stainless steel interior and hinged gabled lid.
* Spares and factory services are available.
* Units constructed to any size to specifications.

CAT: WB.400

MANUFACTURING LABORATORY SUPPLIES LIMITED

P.O. Box 2997,
WELLINGTON.

Five years ago most labs in U.S.A. were still using other thromboplastins.

Today more than 80% have changed to Simplastin®. *Why?*

Such changes are not made lightly.
Each laboratory had its reasons:

Dependability Simplastin's reproducibility from vial to vial and lot to lot is *guaranteed*. We make sure of reproducibility by rigidly controlling such factors as particle size and number, pH, ionic strength, moisture content, temperature stability. We standardize Simplastin against normal and dicumarolized plasmas, against whole and dilute Diagnostic Plasma Warner-Chilcott and against other lots of Simplastin. For accuracy in prothrombintime determinations, the thromboplastin *must* give reproducible results. Simplastin always does.

Purity To laboratories that used to use *liquid* commercial thromboplastin preparations, this has been a significant consideration. (To stabilize thromboplastin in suspension, the manufacturer must use a preservative. Preservatives commonly used for this purpose are phenol (carbolic acid) and formaldehyde—both enzyme poisons that can make prothrombin times less reliable, especially with patients on anticoagulants.)

Convenience For laboratories that once made their own thromboplastin or extracted it from dried brain preparations, this has proved to be an important advantage. Adding water to Simplastin takes a lot less time and trouble than the do-it-yourself procedures — and you couldn't make a finer thromboplastin by any means.

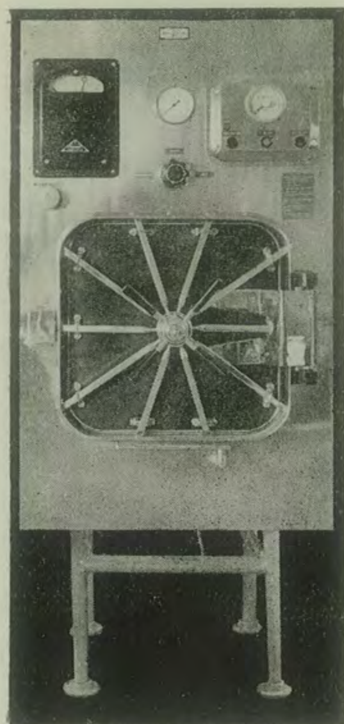
Economy Thanks to the no-waste vials (6 and 20-determination size, to match your laboratory load), the skilled time saved by simple reconstitution, and the less frequent calls for "repeats", many laboratories find that their cost per test is lower with Simplastin.

"It's the standard" Simplastin has become the standard thromboplastin in most coagulation research laboratories, and wherever results must be readily comparable to those obtained in other laboratories.

These are typical reasons why so many laboratories have changed to Simplastin. And why very, very few have changed again.

Like the finest product in any field, Simplastin is flattered by many imitators. They come and go. Even more gratifying is that year after year more and more laboratories, and more and more patients, benefit from our continuing efforts to make Simplastin the finest thromboplastin available.

William R. WARNER and Co. Ltd.
AUCKLAND



MERCER
Bacteriological Autoclave
(Rectangular Type)

These rectangular, single-jacketed units are ideal for Bacteriological Work, in that all chamber space is working space. Type illustrated is fitted with flush panel, mounting adjustable thermometer and adjustable timer . . . both being integrated to permit pre-setting by means of keys, of both temperature and time, taking the guesswork out of all types of media sterilising. Any temperature may be selected up to the equivalent of 30lb pressure for any length of time required up to 60 minutes. Push-button control, light stage indicator.

mercer *SINCE 1884*

Christchurch: J. Mercer and Sons Ltd. Head Office: Moorhouse Avenue. 'Phone 69-679.
Wellington: 10 Vivian Street. 'Phone 53-410.
Auckland: 36 Carr Road, Mount Roskill. 'Phone 558-054 (3 lines).
Otago and Southland: Home Heating Supplies Co. Ltd., 17 St. Andrew Street, Dunedin. 'Phone 70-052.

B.4

GURR'S

REGD. TRADE MARK

ESTABLISHED 1915

STAINS AND REAGENTS FOR MICROSCOPY

Orcein
Silver Proteinate
Fast Cyanine
Coomassie Blue
Evans Blue
Giemsa Stain R66
pH Indicators
Buffer Tablets
Immersion Oils

Mounting Media
Embedding Waxes
Aquax
Plastic Staining Vessels
Embedding Baths and Ovens
Autoclaves
Slide Storage Equipment
Microscope Lamps

"BIOLOGICAL STAINING METHODS"

by George T. Gurr

The seventh edition of this popular and practical manual. Most comprehensive publication of its kind.

Price 7/- (N.Z.) Post Free.

Agents for

DANOCHEMO VITAMIN ASSAY MEDIA

SERVA ION-EXCHANGE CELLULOSES

GEORGE T. GURR LTD.

136/144 NEW KINGS ROAD, LONDON, S.W.6

Cables: Microstain, London, S.W.6.

WHY NOT HAVE THE BEST?

Leitz

MICROSCOPES

ORTHOLUX — LABORLUX — SM
with
FLUORESCENCE — XENON ILLUMINATION

PHASE WITH LIGHT FIELD OR DARK FIELD OBSERVATIONS.

MICROSCOPES AND OPTICAL TESTING INSTRUMENTS FOR ALL PURPOSES.

PROJECTION APPARATUS OF ALL TYPES.

MICROPHOTOGRAPHIC APPARATUS.

CRYSTAL CLEAR IMAGES AT ALL MAGNIFICATIONS.

TECHNICON

AUTO-ANALYSERS

"THE BLACK MAGIC OF CHEMISTRY"

E. C. LACKLAND & CO. LTD.

BLEDISLOE STREET — AUCKLAND, C.1

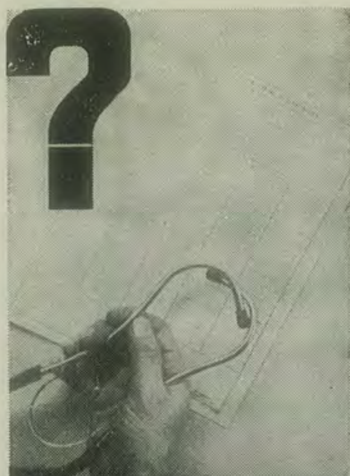
P.O. Box 5284. Telephone 20-136.

The
UNICORN

Credited in folklore with miraculous healing properties the Unicorn is the world-wide symbol of Burroughs Wellcome and Company, manufacturers and suppliers of the highest quality pharmaceuticals since 1880.

BURROUGHS WELLCOME & CO
NEW ZEALAND LTD

AUCKLAND CHRISTCHURCH



what he doesn't know can hurt

Are you testing **gram-negative organisms** routinely for sensitivity to

GRAM-NEGATIVE SPECIFIC ANTIBIOTIC

COLY-MYCIN INJECTABLE

colistimethate sodium

for urinary, respiratory, surgical, wound, burn and blood stream infections

- primarily bactericidal against most gram-negative pathogens — especially Pseudomonas and **E. coli** (not recommended for Proteus)
- exceptionally safe when used as recommended (exercise caution in presence of renal impairment)
- rarely induces bacterial resistance
- therapeutic blood and urine levels rapidly attained

Please remember: Since Coly-Mycin (colistin) is a polypeptide antibiotic, a clear zone of inhibition, regardless of size, indicates sensitivity — usually high sensitivity.

Sensitivity discs are available from your regular suppliers, from this office—free of charge, or your Warner-Chilcott representative. Also, have you seen the 4½ minute bacteriology film on Coly-Mycin (colistin)? Ask your Warner-Chilcott representative about it the next time he calls.

Side Effects: Occasional reactions such as circumoral paresthesias, nausea, dermatitis, drug fever, transient vertigo, and dizziness have been reported and usually disappear upon discontinuance of drug or reduction of dosage.

Precautions: Exercise caution in renal impairment. Transient elevations of BUN have been reported. As a routine precaution blood studies should be made during prolonged therapy.

William R. WARNER and Co. Ltd
AUCKLAND

At Your Service!

WITH . . .

"PYREX" Brand Glassware

"ANALAR" Chemicals

"E-MIL" Graduated Glassware

"WHATMAN" Filter Papers

"ZEAL" Thermometers

. . . and a general range of laboratory requisites always in stock.

WE ARE BOOKSELLERS!

Technical Books from any source available — ex stock or on indent

KEMPTHORNE PROSSER & CO'S.,

NEW ZEALAND DRUG CO. LTD.

Surgical, Dental, Scientific and Fancy Goods Showroom . . .

378 GREAT KING STREET

DUNEDIN

P.O. Box 319. Telephone 77-262

Wild M 11

**The new,
very versatile microscope**

Ideal for instruction and laboratories, for students of medicine and natural science. The basic equipment can be completed any time, up to a small research microscope, according to the " building-block principle."

A Swiss precision instrument equipped with the famous Wild micro-optics. Modern packing in a metal container.

Please write for the pamphlet with all the technical details from the optical works of Wild Heerbrugg Ltd., Heerbrugg / Switzerland

WILD
HEERBRUGG

Full guarantee for prompt service and reliable delivery of spare parts through Swiss firms of world renown.

Sole N.Z. Agents:

DENTAL & MEDICAL SUPPLY CO. LTD.

AUCKLAND - WELLINGTON - CHRISTCHURCH - DUNEDIN

"It's accurate, Doctor. We ran normal and abnormal Versatol® controls at the same time, at several levels, as part of our new quality control programme."

Laboratory heads everywhere are answering clinicians with greater assurance, thanks to modern methods and materials for routine, daily quality control. The Versatol series, currently available comprise:-

Versatol: normal reference standard for 12 serum constituents

Versatol-A: abnormal reference standard for 16 constituents.

Versatol-A Alternate: alternate abnormal reference standard for 16 constituents.

Versatol Paediatric: reference standard for infant serum: normal for 13 constituents, abnormal for bilirubin (20mg./100ml.)

Details of the Versatol series, or information concerning the full range of Warner-Chilcott diagnostic reagents is available from the N.Z. agents on request:

William R. WARNER & Co. Ltd.

P.O. Box 430 Auckland

Here's why B-D Vacutainer Specimen Tubes make blood collection easier...simpler

Specially designed B-D Sterile Disposable VACUTAINER Needles attach quickly and easily...have Microlance points for greatest sharpness ever.

Color-coded stopper is designed for easy cleaning...simple removal. Unique construction prevents vacuum loss.

Reusable plastic holder does not require sterilization after use...assembled unit handles like an aspirating syringe.

VACUTAINER Specimen Tube produces consistent blood specimen volume... permits centrifuging without transfer to another tube. Available with a wide range of additives.

B-D Vacutainer Blood Collecting System functions as an aspirating syringe... speeds work...cuts cost

B-D

BECTON, DICKINSON AND COMPANY, Rutherford, New Jersey

Sole Agents in N.Z.: **Biological Laboratories Ltd.**

Private Bag, Northcote, Auckland.

Contents

TRAINING AND EXAMINATIONS
Guest Editorial - - - - - - - 98
LACTIC DEHYDROGENASE AND THE APPLICATIONS OF ITS ESTIMATION IN THE ROUTINE CLINICAL LABORATORY
T. E. Brown - - - - - - - 100
A MICRO DROP TECHNIQUE FOR THE TITRATION OF ANTI-STREPTOLYSIN O IN HUMAN SERUM
B. F. Dawkins - - - - - - - 115
AN IMPROVED COLORIMETRIC METHOD FOR THE ESTIMATION OF LIPASE IN SERUM USING PHENYL LAURATE AS A SUBSTRATE
A. G. Wilson - - - - - - - 119
ACCURACY AND QUALITY CONTROL IN CLINICAL CHEMISTRY
Adrienne Ramsay - - - - - - - 125
THERMO-ELECTRIC COOLING FOR RESEARCH, HOSPITALS AND INDUSTRIAL LABORATORIES 130
SELECTED ABSTRACTS - - - - - 133
THE LIBRARY. (List of Current Acquisitions) - - 137
BOOK REVIEWS
Steroid Determinations - - - - - 138
Textbook of Microbiology - - - - 139
ANNUAL CONFERENCE (1964) - - - - 140
MINUTES OF THE TWENTIETH ANNUAL GENERAL MEETING - - - - - 142
PAPERS READ AT THE 1964 ANNUAL CONFERENCE - - - - - - - - 145
COUNCIL NOTES - - - - - - - 147
BRANCH REPORTS - - - - - - - 148
AUCKLAND PRIZEGIVING 1964 - - - 149
LETTERS TO THE EDITOR - - - - 151
DIRECTIONS FOR CONTRIBUTORS - - - 155
ANNOUNCEMENTS
Vacancies - - - - - - - - 99, 150
Associateship Diplomas - - - - - 114
Annual Subscriptions - - - - - 118
Changes of Address - - - - - 129
Annual Conference 1965 - - - - 146
INDEX TO VOLUME 18 - - - - - 156

PRINTED BY THE EVENING STAR CO. LTD.,