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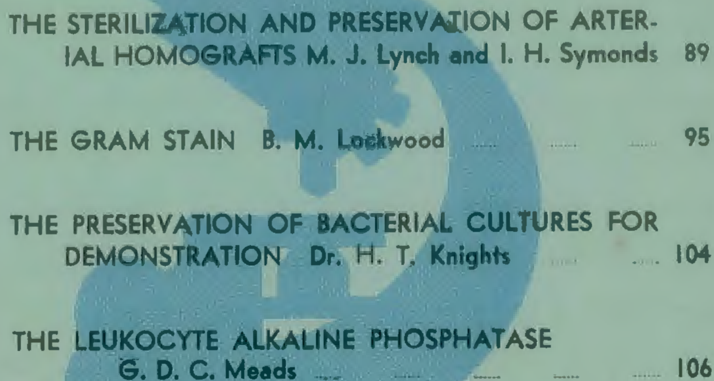
JOURNAL OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

CONTENTS



THE STERILIZATION AND PRESERVATION OF ARTERIAL HOMOGRAFTS M. J. Lynch and I. H. Symonds 89

THE GRAM STAIN B. M. Lockwood 95

THE PRESERVATION OF BACTERIAL CULTURES FOR DEMONSTRATION Dr. H. T. Knights 104

THE LEUKOCYTE ALKALINE PHOSPHATASE G. D. C. Meads 106

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ADDRESSES

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THE STERILIZATION AND PRESERVATION OF ARTERIAL HOMOGRAFTS

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Since there is an ever-increasing demand for sterile arterial homografts in the field of vascular surgery and since the most likely source of such tissues is from autopsy material, a simple and inexpensive method of sterilizing and preserving these tissues is needed. A homogenous artery bank has been in operation in this hospital since 1956, and it is the purpose of this paper to describe the techniques used for the sterilization and preservation of these arteries.

As contamination of arteries such as the aorta and the iliac arteries is rapid and may take place within as little as one hour of death[a], collection of sterile arteries is practically impossible. Therefore, methods of efficient sterilization must not overlook the hazards of possible viral, parasitic and mycotic infections as well as bacterial infection. Obviously, it is also essential that arteries collected for storage in the bank must be free from any disease.

With the aim of complete sterilization in view, Lo Grippo et al[b] found that after studying the effects of several hundreds of chemical agents, few showed any virucidal activity without the production of toxic substances. Of the agents screened, beta-propiolactone was found to be the most suitable sterilizing agent.

Lo Grippo[b] tested the ability of beta-propiolactone to inactivate the following—viruses, fungi and bacteria:

Viruses	Fungi	Bacteria
Lymphocytic choriomeningitis	Aspergillus niger	E. coli
Eastern equine encephalomyelitis	Microsporum audouini	Bacillus proteus
Coxsackie	Microsporum canis	Pseudomonas aeruginosa
Poliomyelitis (Lansing)	Trichophyton rubrum	Salmonella typhi
Rabies	Trichophyton mentagrophytes	Shigella dysenteriae
Influenzae		Streptococcus haemolyticus
		Enterococcus
		Staphylococcus aureus
		Bacillus globigii (desiccated spores)

He found that a 1% solution of beta-propiolactone is 80%, 66%, 50% and 25% in excess of the concentration required to kill the most resistant bacteria, viruses, fungi and spores respectively. With these results obtained in vitro, the drug was tested in the sterilization of contaminated arteries obtained from and transplanted into dogs[c]. The results of these experiments were so encouraging that banks of human arteries obtained from autopsy patients and sterilized with beta-propiolactone were soon established.

Beta-propiolactone

Beta-propiolactone is a liquid that is miscible with water to the extent of 37% by volume at 25°C. It is stable in its concentrated form but it is unstable in aqueous solution. In the presence of certain acids, alkalis and inorganic salts, explosive polymers can be formed. Although in its concentrated form, beta-propiolactone is toxic to tissues, the diluted form at room temperature hydrolyses rapidly, producing non-toxic degradation products. Because of these characteristics, a 1% solution is safe to handle, kills all infective agents, causes little denaturation and then hydrolyses itself.

LABORATORY PROCEDURE

1. *Collection of Arteries:*

Preferably, the donor's age should be forty years of age or under, and only those patients with arteriosclerosis or neoplastic disease, or with tuberculosis or any other specific infective diseases are avoided. Arteries should be obtained within a maximum of twenty-four hours of death. Gross contamination is avoided and the whole aorta with the iliac and femoral arteries are removed as far as the origin of the popliteal arteries.

The abdominal aorta is divided at the level of the renal arteries and the iliac arteries are divided at the origin of the femorals. The resulting four pieces, after washing in sterile saline, are placed in four sterile pyrex tubes (36 x 270 mm) and stoppered with sterile cotton wool plugs. The tubes are stored in the deep freeze refrigerator until they are trimmed, measured and sterilized. Aerobic, anaerobic and fungal cultures are prepared from the saline washings.

With unnecessary contamination being avoided, each specimen is allowed to return to room temperature, washed in sterile saline and laid on a sterile towel where all the fat and connective tissue is cut off. The arterial branches are cut short (3-4 mm.) but are not ligated. A drawing is made to indicate the shape of the graft and measurements of the length and diameters are taken and enter-

ed in the bank register. Remarks are added, indicating any information considered to be of use to the surgeon. These points are especially necessary when freeze-drying is the method of preservation, for dehydration causes shrinkage. Directions for reconstitution of the graft are also included in the register.

2. *Sterilization by Beta-propiolactone:*

Just prior to sterilization a 10% solution of beta-propiolactone in ice cold water is prepared (2.2 ml. concentrated beta-propiolactone diluted to 25 ml). The mixture is shaken vigorously to allow the drug globules to go into solution. This solution is rapidly diluted to a 1% solution with a saline-bicarbonate buffer. The buffer solution is necessary because hydrolysis products of propiolactone tend to lower the pH. A solution of 0.2M. sodium bicarbonate in physiological saline solution is sufficient to maintain the pH. between 6.5 and 7.0 during incubation.

The arteries contained in sterile pyrex tubes are immersed in the 1% solution of beta-propiolactone and are incubated for 2 hours at 37°C. During incubation, sterilization is effected and the reagent is completely hydrolysed to non-toxic products. It must be remembered that these products of hydrolysis are no longer bactericidal and that the arterial specimens must now be handled aseptically. The arteries are removed from the sterilizing solution, washed once in sterile saline, and placed in new sterile pyrex tubes that have their open ends closed with sterile gauze covers.

Cultures are prepared from the saline washings and the sterile arteries are returned to the deep-freeze refrigerator to await freeze-drying. If the cultures reveal any contamination, the sterilization procedure must be repeated.

3. *Freeze-drying:*

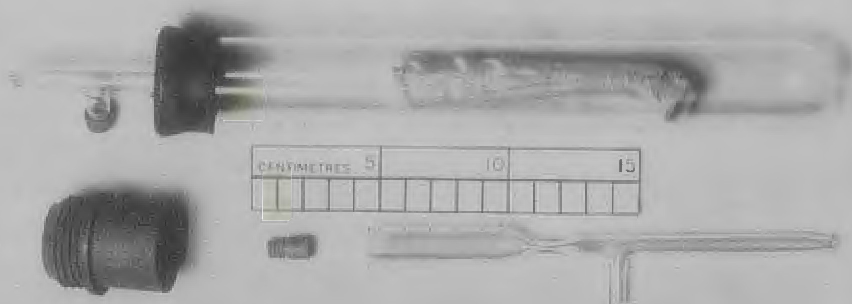
The freeze-drying apparatus is of a standard design with primary and secondary chambers, refrigeration unit, condenser and a manifold assembly. The refrigeration unit maintains the temperature of the condenser plates at —45°C. This is necessary to prevent the primary pump from becoming swamped. In order to utilize latent heat in obtaining more efficient drying, the primary chamber is fitted with a coil heater which allows the temperature of the chamber to rise gradually to 30°C. In the primary drying stage, using a vacuum of 1.5 to 0.5 mm. mercury measured on a Pirani gauge, about 98% of the water in the tissue is removed.

For secondary drying, a vacuum of 0.1 to 0.01 mm. mercury is required. The small amount of water remaining in the tissue is removed, and is absorbed by a phosphorus-pentoxide moisture trap.

Primary Drying: The refrigerator unit is turned on and when the temperature of the condensing plates reaches —45°C. the tissues are removed from the deep freeze refrigrator and placed in the primary chamber. The chamber is sealed and the vacuum pump started. The primary drying lasts 30 hours.

Secondary Drying: After primary drying, the grafts are placed directly in the secondary chamber, and the system evacuated to a pressure of 0.01 mm. mercury. A further 30 hours drying is carried out in this chamber.

The manifold assembly allows the tubes containing the arteries to be sealed off in a vacuum. The pyrex tube is removed from the secondary chamber and is fitted with a sterile rubber stopper containing suitable attachments to allow evacuation of the tube. The rubber stopper is a "Subaseal" artery graft closure type 3.B.22A. with a central perforation. A piece of narrow pyrex tubing with a small side arm fitted with a "Subaseal" 3.B.11 is inserted into the central perforation of the large stopper. The opposite end of the pyrex tube is filled with cotton wool and the whole attachment is sterilized by autoclaving. The flange of the sterile stopper may require to be moistened with a little sterile liquid paraffin in order to ease the insertion into the mouth of the tube containing the artery (fig. 1). The manifold appartus is connected to the second-



ary vacuum pump and the Pirani vacuum gauge. The artery tube with the head attachment is connected to the manifold apparatus is connected to the secondary vacuum pump and the Pirani

vacuum gauge. The artery tube with the head attachment is connected to the manifold and the vacuum pump is started. Since the arteries no longer contain moisture, only two to three hours under these conditions are necessary to ensure complete evacuation.

The artery tubes are sealed by heating the small pyrex tubes above the side arm using a cross-fire burner burning coal gas and oxygen. The sealed tube is tested for a vacuum with a high frequency spark tester.

If the aerobic and anaerobic cultures taken from the washings after sterilization with beta-propiolactone reveal no contamination, the arteries are now labelled and placed in the bank and their details are entered in the artery register. These arteries may be kept at room temperature for several years.

4. *Reconstitution:*

The surface of the subaseal on the short side arm is sterilized with either iodine or alcohol and the needle of a sterile blood transfusion giving set attached to a bottle of sterile saline solution is plunged through it. As a safeguard against infection in the operating theatre, 2 gms. of streptomycin and 2,000,000 units of penicillin are added to the reconstituting fluid. If desired, heparin may also be added. At least half an hour must be allowed for the graft to reconstitute.

RESULTS:

To date (11.11.60) 38 arteries have been processed in the manner described, and 15 have been used successfully in transplants. Below is a table which shows the relative numbers of homografts to nylon and dacron grafts performed at this hospital up to 11.11.60.

	Total	Nylon Dacron or Orlon	Freeze dried Homografts
Aortic	6	3	3
Iliac or Femoro-popliteal	18	6	12
Totals	24	9	15

SUMMARY:

1. A method for the sterilization by beta-propiolactone of human arterial transplants obtained at necropsy is described.

2. The techniques of freeze-drying and their application to tissue graft storage are presented.

3. A summary of arteries sterilized and used in transplants in the Wellington Hospital is given to demonstrate the efficacy of principles described.

ACKNOWLEDGEMENTS:

The authors are indebted to Mr J. H. North, Medical Superintendent of Wellington Public Hospital and Dr. J. O. Mercer for permission to publish this paper and to Dr. J. Hopkins for his assistance in preparation.

REFERENCES:

a. Rains A. J. H., et al (1956) The Lancet. Oct. 20.

b. Lo Grippo, G. A., et al (1955) Laboratory Invest. 4.217-231.

c. Trafas, P. C., et al (1954) A. M. A. Arch. Surg. 69:415.

THE GRAM STAIN

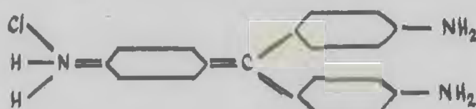
Bruce M. Lockwood
(*Pathology Department, Palmerston North Hospital*)
(Winner Junior Essay Competition, 1961)

The most celebrated use of trapping agents in microtechnique was made by Christiaan Gram in 1884. Gram discovered this technique quite by accident while he was looking for a double staining technique for kidneys containing casts in the tubules. The double dye he used, contained gentian violet to stain the chromatin blue, and iodine, dissolved in potassium iodide solution, to stain the casts brown. The subsequent decolourising with alcohol removed all traces of the stain. He then tried it on other organs to see if it would work and fortunately chose an infected kidney. The result was startling on account of the numerous bacteria being stained an intense purple by the gentian violet. When differentiated with alcohol all the tissues lost their colour. This rendered bacteria more visible than had been the case before. Gram later found that this applied only to some bacteria. Since then the staining technique has had many modifications, but the technique still consists of four main steps.

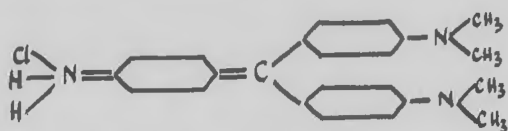
1. *Primary Stain:* The bacterial smear is treated with a dye of the pararosanilin series.
2. *Mordant:* Iodine in aqueous potassium iodide is used to fix the primary stain against decolourisation.
3. *Decolourisation:* The smear is quickly washed in some organic solution to remove excess stain and decolourise gram negative organisms.
4. *Counterstain:* The decolourised organisms are re-coloured with a basic dye usually red in colour to contrast with the primary stain.

CHEMISTRY OF DYES:

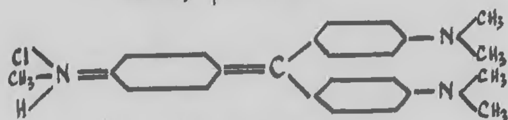
The first artificial dyes were made from aniline and were therefore called "aniline dyes." Aniline is a mono-substitution product of benzene. The oxidation of mixtures of aniline and toluidines by special methods is used in the manufacture of pararosanilin and rosanilin dyes. Pararosanilin dyes are used in the Gram's stain as these have proved more satisfactory. Those more generally used in the technique include methyl violet, crystal violet and gentian violet. Pararosanilin belongs to a group of dyestuffs known as triphenylmethane dyes. This compound has the form of a benzene compound with an amino group attached to the ring.



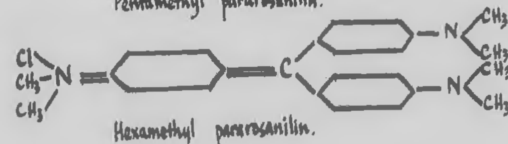
The dye can be progressively deepened in colour by the substitution of hydrogen by radicles. These radicles are generally ethyl, methyl and sometimes phenyl groups. When the methyl groups are attached onto the amino radicles, the methyl violets are produced. These dyes consist of various mixtures of tetra—, penta—, and hexamethyl pararosanilin.



Tetramethyl pararosanilin.



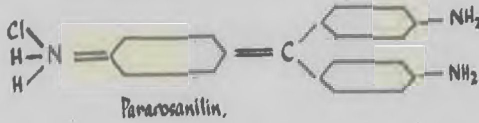
Pentamethyl pararosanilin.



Hexamethyl pararosanilin.

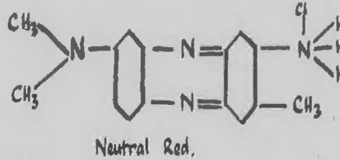
The depth of colour progresses from the lightest tetramethyl pararosanilin to the deepest, crystal violet. Gentian violet, sometimes used in the gram stain is a poor mixture of the violet pararosanilins, giving it a shade of colour about as deep as methyl violet. Crystal violet is considered the most satisfactory dye for the gram technique since it comes the closest to a definite chemical compound.

Basic fuchsin, which is generally used in the counterstain, is the simplest of rosanilin dyes. This dye differs from the methyl violets in that the amino groups are not substituted or methylated. The principal constituent of commercial basic fuchsin is pararosanilin (magenta O). The dye usually contains rosanilin and magenta II. Basic fuchsin is generally present as the acetate and sometimes as the chloride.



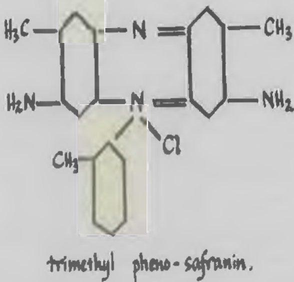
Pararosanilin.

Neutral red and safranin are also commonly used as counter-stains. Neutral red is a derivative of phenazin and belongs to the group of dyes, the azur group, as does safranin. Neutral red is a weakly basic dye, yellow in alkaline and red in acid solution. The dye is also a weak nuclear stain and has the formula:

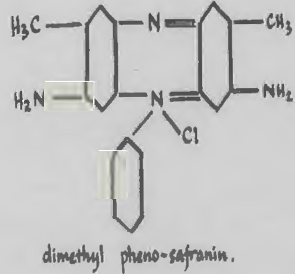


Neutral Red.

Safranins are divided into two groups, the benzo-safranins and the naptho-safranins. The safranin used in the Gram Stain belongs to the latter group and is a mixture of di-methyl and tri-methyl pheno-safranin.

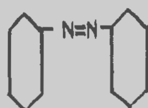


trimethyl pheno-safranin.



dimethyl pheno-safranin.

For the compounds listed above to display the properties of a dye, they must contain two elements; an auxochrome and a chromophore group both being attached to the benzene ring. The auxochrome determines the acidic or basic nature of the dye. The group may be acidic, containing the acidic hydroxyl group (—OH), or basic, containing the basic amino group (—NH_2). The chromophore group or groups, give the dye its particular colour. The acid chromophore includes the nitro group (—NO_2) and the quinoid ring. The basic chromophore groups include the azo linkage (—N═N—) where a benzene ring is attached to each nitrogen atom.



MODIFICATIONS:

Primary Stain: The dyes generally belong to the group of dye-stuffs; the pararosanilins. Bartholomew and Mittwer[d] made a survey by testing all the major dyes for their suitability in the Gram Stain. The most suitable substitutes for crystal violet, which is the generally accepted primary stain, are brilliant green, malachite green, basic fuchsin, ethyl violet and methyl violet. All of these dyes are basic. The acid dyes tested were either useless or inferior in reaction. Some workers claimed a lack of stability in the primary stain, due to the slow formation of a precipitate. Huckers[q] used ammonium oxalate in the stock primary stain and this he claimed considerably increased the stability of the stain. Kopeloff and Beerman[w] added 5% sodium bicarbonate to the primary stain giving a pH of 9.3. This was added to counteract the sometimes acid state of organisms and to give a more intense gram positivity.

Mordant: Iodine is the almost universal trapping agent. It is seldom used in its blue molecular form, but it is nearly always dissolved in aqueous potassium iodide solution giving a brown tri-iodine complex. Iodine is not the only mordant possible, reagents such as potassium permanganate, bromine, picric acid and metallic iodides have been found in some cases to be satisfactory but inferior to the iodine complex. Some formulae contain sodium bicarbonate or sodium hydroxide in the iodine solution (Atkins[a]). but this is not critical and seems to be an individual preference.

Decolouriser: A number of organic reagents for this critical step can be used. The principle reagents used will be treated individually.

Alcohol: This reagent can be used at varying strengths. The addition of water to the alcohol increases the power of the decolouriser to take up the dye precipitate into solution and thus increases the rate of decolourisation of gram positive organisms. Absolute and 95% alcohol give the most satisfactory results as they allow a longer time for differentiation than the other dilutions. 75% alcohol decolourises gram positive organisms almost as rapidly as gram negative organisms, while 50% decolourises both type simultaneously.

Alcohol and Acetone: This reagent has an advantage as the mixture increases the rate of decolourisation of gram negative organisms and slows the rate of decolourisation of gram positive organisms.

Acetone: Gram negative organisms are decolourised five to ten times as fast as with absolute alcohol and gram positive organisms much more slowly. 80% acetone has the advantage over 95% alcohol in that it reduces the time for the decolourising process and gives a longer time between the decolourisation of gram positive and gram negative organisms.

Counterstain: This dye must not have the property of being able to displace the primary stain or be so concentrated as to mask the primary stain. Usually dilute solutions of basic fuchsin, safranin, neutral red and carbol fuchsin are used. The choice of stain for this step is not critical, although it is usually of the red range of colours, to contrast it with the blue which is most commonly used in the primary stain.

THEORIES:

Gram in his original work did not attempt to explain the mechanism of the staining procedure he discovered. Two early attempts were made, Unna in 1887 and Eissenberg in 1909 trying to explain the reaction on a chemical basis. Since then intensive research has brought out three main theories which will be dealt with under:—

1. Cell Wall and Permeability.
2. Isoelectric Point.
3. Chemical Theories.

1. *Cell Wall and Permeability:*

According to Churchman[m] all gram positive cells contain a gram positive external cortex surrounding a gram negative internal medulla. He experimented with Bacillus anthracis and converted it to a gram negative state. This conversion was accompanied by a 40% reduction in size. A 50% reduction was also noted with yeast. When these organisms were converted to the gram negative state, there was a loss in weight, which, he claimed, showed they had lost their gram positive cortex. Bartholomew and Umbreit[h] also noted a reduction in the size of cells which were treated with ribonuclease. They correlated this phenomenon with that of Churchman's. Bartholomew and Mittwer[e] converted Bacillus subtilis to the gram negative state without noticing a decrease in size. They explained Churchman's conclusion as a result of using

acriviolet. According to them the acriviolet caused some of the cytoplasm to be expelled thus allowing room for shrinkage. Kaplan et al[w], Bartholomew et al[f] and Burke et al[k], concluded that in Gram positive cells iodine served to form a dye-iodine-precipitate in the cell. Because gram positive cells were less permeable to iodine in alcohol Kaplan[t], Bartholomew[f] and Burke[k] and acetone Knaysi[x] than gram negative cells, the complex dissolved out of the gram positive cell at a slower rate than the gram negative cell. They proposed that the mechanism of the gram stain is entirely one of membrane permeability. They also claimed that this theory is supported by the fact that chrushed gram positive cells (the membrane being thus disrupted) stain negatively. Studying the result of crushed cells, Burke and Barnes[i k] showed that the protoplasm was expelled leaving the empty sac. Studying the staining of whole and crushed cells led them to state that the protoplasm of the cell plays no part in the gram retention and bacteria are gram positive, negative or variable according to the permeability of the cell wall.

2. *Isoelectric Point:*

Stearn and Stearn[ee] determined the isoelectric points (the pH at which the cell has no net charge) on the basis of the cells affinity for acid and basic dyes at various pHs. From the retention of various cells for basic dyes they showed that gram positive organisms had a slightly lower isoelectric point than gram negatives. Thompson and Dubos[hh] observed that when pneumococci were killed at pH 4.2-4.4 with acetic acid, they retained their gram positive nature only as they were kept at this reaction. When they were resuspended in a neutral medium, enzymatic action took place changing them to gram negative.

Since the Gram stain is carried out at or near to neutral reaction, Stearn and Stearn[ee] postulate that the gram positive organisms take up more dye and are more firmly united to it. They also state that the lower the isoelectric point the stronger the union between the amphoteric substance and the dye, and thus the greater the difficulty to break this union by decolourisation. The iodine acts by increasing the isoelectric range between gram positive and gram negatives. In the place of iodine any oxidising agent can be used such as those that have been discussed. However this theory has two unexplainable enigmas. Firstly, this theory should account for crushed cells as well as for whole cells, but crushed cells have been shown to stain negatively. Secondly, if iodine is used before the primary stain the differentiation should be successful but, Burke et al[i] and Bartholomew et al[d] have shown that this is not so.

3. *Chemical Theories:*

Some early workers (Dreyer et al[o]) attributed the gram complex to lecithin. They treated Escherichia coli with lecithin and claimed they were made positive. This, however, has been found difficult to reproduce and has received no more support than the early work on fatty acids. (Jobling and Petersen[t]).

Many workers have investigated the possibilities of nucleoproteins as being the explanation. Webb[ii] noticed that when gram positive organisms were rendered gram negative by the action of lysozome, small amounts of reducing substances were liberated from the cells. Henry and Stacey[r] analysed these extruded substances and found them to contain carbohydrates, proteins and magnesium ribonucleate. Jones et al[u] "stripped" the gram positive material from Saccharomyces cerevisiae and the extract yielded polysaccharides and magnesium and sodium salts of RNA. Mitchell and Moyle[aa bb] showed that the removal of RNA from cells made them gram negative, while still retaining DRNA in the negative cycloskeleton. Because of this they hold that DRNA plays no part in the gram complex. The substance that seems to be responsible is magnesium ribonucleate. Many authors have repeatedly shown that cells rendered negative can be made positive again by replating them with magnesium ribonucleate. Henry and Stacey[q r] were able to do this by using magnesium ribonucleate obtained from yeast. Although cells rendered gram negative can be made gram positive again, truly gram negative organisms cannot be made gram positive in this way (Bartholomew and Umbreit[h]) although a small degree of positivity has been obtained with Neisseriae. Some workers claim that ribonucleate on its own is not the cause of gram positivity. Henry et al [q r] believed that the substance is a ribonucleate combined with a protein of a basic type. Small quantities of RNA have been detected in negative cells and Stacey [dd] proposed that the reason for gram positivity was the ratio of RNA to DNA. He showed that in streptococci the ratio RNA/DNA was 8:1 while in gram negatives the ratio was 1:3, but according to Mitchell and Moyle[aa] there is no such relationship.

Fisher and Larose[p] believe that that complex is due to the presence of a sulphydryl group and the disappearance of this group is associated with the change to gram negative. Mitchell and Moyle[bb] made a survey on a small number or organisms and found the distribution of the phosphoric ester XSP to be more or less in accordance with the gram classification, but they were unable to show its presence in Clostridium welchii which is one of the strongly gram positive organisms. Jones et al[u] have claimed a

much lower XSP content in gram positive organisms and state that there is not sufficient evidence to connect the gram complex witht he XSP content. Of the chemical theories the ribonucleates seem to have the most support foı being responsible for the gram complex, but this evidence is far from conclusive.

SUMMARY:

The chemistry of the dyes used in the Gram stain has been presented. The various dyes which can be used in this technique have been discussed and crystal violet has been shown to be the best dye for the primary stain. The different decolourising reagents have been treated similarly as to their ability to remove the primary dye from gram negative organisms while leaving it intact in gram positives. A review of the counterstains has been made and basic dyes of the red range of colours have been shown to be the most suitable.

The theories that have been proposed to explain the mechanics of the Gram stain have been discussed and dealt with under the collective headings:—

1. Cell Wall and Permeability.
2. Isoelectric Point.
3. Chemical Theories.

REFERENCES:

[a] Atkins K. M., (1920), J. Bact., **5,** 321.

[b] Baker J. R., 1958, Principles of Biological Microtechnique.

[c] Bartholomew J. W., and Fiukelstein H., (1958), J. Bact., **75,** 71.

[d] Bartholomew J. W., and Mittwer T., (1950), Stain. Tech., **25,** 103.

[e] Bartholomew J. W., and Mittwer T., (1951), J. Gen. Microbiol., **5,** 39.

[f] Bartholomew J. W., and Mittwer T., (1956), J. Bact., **72,** 337.

[g] Bartholomew J. W., Mittwer T., and Kallman B. M., (1950), Stain Tech., **25,** 169.

[h] Bartholomew J. W., and Umbreit W. W., (1944), J. Bact., **48,** 567.

[i] Bourke V., (1922), J. Bact., **7,** 159.

[j] Burke V., and Barnes M. W., (1928), **15,** 12, J. Bact.

[k] Burke V., and Barnes M. W., (1929), J. Bact., **18,** 69.

[l] Burke V., and Gibson F. C., (1933), J. Bact., **26,** 211.

[m] Churchman J. W., (1929), J. Bact., **18,** 413.

n. Conn H. J., (1946), Biological Stains, 5th Edition.

o. Dreyer G., Scott S. G., and Walker E. W., (1911), J. Path. Bact., **16,** 146.

p. Fisher R., and Larose P., (1952), J. Bact., **64,** 435.

q. Henry H., Stacey M. and Teece E. G., (1954), Nature **156,** 720.

r. Henry H., and Stacey M., (1943), Nature **151,** 671.

s. Hucker G. T., (1921), J. Bact., **6,** 395.

t. Jobling J. W., and Peterson W., (1914), J. Exp. Med., **20,** 452.

u. Jones A. S., Rizui S. B. and Stacey M., (1958), J. Gen. Microbiol., **18,** 597.

v. Jones A. S., Muggleston P. W. and Stacey M., (1950), Nature **166,** 650.

w. Kaplan M. L. and Kaplan L., (1933), J. Bact., **25,** 309.

x. Knaysi G., Elements of Bacterial Cytology.

y. Kopeloff N. and Beerman P., (1922), J. Infect. Diseases, **31,** 480.

z. Lamanna C., and Mallette M. F., (1954), J. Bact., **68,** 509.

aa. Mitchell P., and Moyle J., (1954), J. Gen. Microbiol., **10,** 533.

bb. Mitchell P., and Moyle J., (1950), Nature, **166,** 218.

cc. Oginskey G. L. and Umbreit W. W., An Introduction to Bacterial Cytology.

dd. Stacey M., (1948), Symposia Soc. Exp. Biol., **1,** 86.

ee. Stearn A. G., and Stearn G. W., (1927), J. Bact., **14,** 349.

ff. Tanner F. W., (1933), Practical Bacteriology 2nd. Ed.

gg. Thimann F. V., (1955), Life of Bacteria.

hh. Thompson R. H., and Dubos R. J., (1938), J. Biol. Chem., **125,** 65.

ii. Webb M., (1948), J. Gen. Microbiol., **2,** 260.

THE PRESERVATION OF BACTERIAL CULTURES FOR DEMONSTRATION

Dr. H. T. Knights
The National Health Institute, Wellington.

Four months ago the National Health Institute was required to furnish cultures of bacteria illustrating staphylococcal phage typing growth of air borne organisms and coagulase positive staphylococci on various media.

The readiness with which such cultures become contaminated and the media dried and cracked prompted an experiment with formalin vapour as preservatizing agent.

The formalin vapour was generated by the action of potassium permanganate upon 40% (B.P.) formalin liquid. The method employed was as follows:

40-50 ml. of the formalin solution were placed in the base of a Petri dish which was then placed on the shelf of a Scheibler dessicator jar.

3-5 grams of potassium permanganate were placed in the formalin solution and a culture of the organisms to be preserved placed growth downwards over the jar and exposed to the action of the formalin vapour for 3-5 mins.

The size of the dessicator quoted can cope with the 5½ inch diameter Petri dish and if a Perspex mask be turned with a slightly bevelled outer edge to fit the internal diameter of the dessicator jar, and with an internal diameter of 3 inch, the apparatus can deal with the 3½ inch diameter Petri dish. After exposure to the formalin vapour the base of the dish is removed and the lid applied and secured by short strips of cellophane.

The media to employ with most success are the clear types; blood agar turns a dirty chocolate brown.

Further experiments are being carried out using the slow generation of formalin vapour from paraform tablets but the foregoing method commends itself by its quickness.

RESULTS:

The original cultures after 4 months at room temperature are free from contamination and the media moist and uncracked, while the details of the colonies can be picked out. Phage typing plates so notoriously difficult to preserve for more than 2 or 3 days are similiarly clear after 4 months.

Such a method commends itself to all teaching institutions for long term demonstrations and to the smaller hospital laboratories where the preparation of cultures for nurses' lectures not only takes up valuable time but the requisite cultures may be difficult to obtain upon demand.

ACKNOWLEDGMENTS:

My thanks are due to Dr. H. B. Turbott, Director General of Health, for permission to publish this article, and to Mr J. Mahn, instrument maker, Wellington Hospital, for turning the Perspex masks.

THE LEUKOCYTE ALKALINE PHOSPHATASE

G. D. C. Meads

(*Pathology Dept., New Plymouth Hospital*)

New and efficient methods for the separation of leukocytes from blood make possible the estimation of leukocyte constituents and enzyme activity by chemical means. The biochemist is able now with new and adequate tools to provide a pure leukocyte preparation with little red cell or platelet contamination.

Grafe[a] in 1911 performed the first studies in leukocyte respiratory activity. Various other workers investigated the conversion of glucose into lactic acid by leukocytes. The pattern of leukocyte biochemical, metabolic, enzymatic and non-enzymatic behavious began to emerge into a formula consistent with various diseases.

In the expanding field of enzymatic and metabolic chemistry, the leukocyte is included among the many body tissues studied. The returns from haematological investigations using special morphologic staining methods are yielding less fruit.

The leukocyte offers itself as a nucleated human tissue showing most of the biochemical and enzymatic activity of other body tissues. In all types of disease the patient is not harmed or put to undue discomfort should these free-flowing cells need to be repeatedly examined.

Many workers in many laboratories have studied the leukocyte from normal and diseased subjects. Among many of the components measured are phosphatases[d], esterase and lipase, glycogen, cell amino-acid levels, transaminase, sulphydryl constituents, enzymatic activity of beta-glucuronidase, histamine location, glycolysis and respiration of the cell. Of the enzymes found in leukocytes the phosphatases have been the most studied and found to be the most useful, although Valentine and his colleagues suggest that bizarre haematological disorders should be studied by the three leukocyte biochemical parameters, histamine, glycogen and phosphatases[f].

The presence of phosphatase in leukocytes has been recognised since the early work of Roche and has been confirmed by Gomori[j]. by Fell and Danielli and by Waestein using histo-chemical techniques. Quantitative studies were made by Haight and Rossiter and Valentine[a] [j].

By using both biochemical and histochemical procedures it is possible to demonstrate in morphologically identical cells differences in the pattern of enzymatic activity. Both methods have some advantages. Cytochemical methods do not require venous blood or

separation of leukocytes from platelets and red cells. But solutions and stains are no less critical than those used in the biochemical quantitative test and control blood must be run in parallel. Phosphatase activity is expressed as absent, weak or strong. Some workers "score" one hundred consecutive neutrophilic segmented and band cells 0 to 4 according to the degree of positive staining[i] and this may be assessed differently by other workers. The quantitative procedure involves the separation of leukocytes from venous blood but gives a result in terms of standard units and should not vary appreciably from worker to worker.

It has been stated that the alkaline phosphatase is contained only in the cytoplasm of the segmented and band form neutrophils and in peripheral blood is shown poorly in about one third of the neutrophils histochemically. The alkaline phosphatase found in the leukocytes appears to be identical in behaviour to the alkaline phosphatase found in many body tissues. Although cytoplasmic[d] its level in the leukocyte is not discernably related to the serum alkaline phosphatase.

As phosphomonoesterases, the acid and alkaline phosphatases are readily estimated quantitatively in suitable incubation systems having optimum hydrogen iron concentrations of 5.0 and 9.9 respectively. Saponin is employed as a cell membrane disrupting agent aiding enzyme contact with the buffered substrate, beta-glycero-phosphate in the presence of a metal activator, magnesium.

In the final spectrophotometric measurement of the inorganic phosphorus liberated in the acid medium normal leukocyte populations on the average hydrolyse 21.9 mg. of phosphorus per hour per 10^{10} cells at 37°C and 25 mg. in the alkaline incubation system. The normal range variation is greater in the case of alkaline phosphatase than in acid phosphatase. Both enzymes appear to be low in the lymphocytes of chronic lymphatic leukaemia and in the blast cells of acute leukaemia. Acid phosphatase does not vary appreciably in neutrophilic leukocytosis of infectious or non-infectious origin[h]. It averages very modestly above the normal mean in chronic myelocytic leukaemia and in polycythaemia vera with leukocytosis.

By comparison alkaline phosphatase varies considerably under the impact of disease[h] and pregnancy([g]. In chronic myelocytic leukaemia the unit leukocyte alkaline phosphatase is lowered to an average of about 4.0 mg. per 10^{10} cells per hour at 37°C. Haemoblast cells[f] are poor in phosphatase and increased numbers decrease the alkaline phosphatase per 10^{10} leukocytes. With this in mind we might say that this reduction of splitting capacity is due

to the increase of the less mature granulocytes present in this disease. Morphological immaturity indistinguishable from chronic myelocytic leukaemia may be seen in polycythaemia vera and in certain myeloproliferative syndromes.

Patients with well proved polycythaemia vera may show such abnormalities and the possibility of transition to chronic myelocytic leukaemia may be considered. The shift left may be from moderate to severe with total leukocyte counts of 15,000 per cmm. to 90,000 per cmm. The leukocyte alkaline phosphatase is strikingly abnormal in polycythaemia. The mean value[c] of 77 mg. of phosphorus liberater per 10^{10} cells per hour is three times greater than that of normal subjects and twenty[h] times greater than the mean value found in cases of chronic myelocytic leukaemia. These differences appear to be independent of therapy[i] or leukocyte differential and like other biochemical behaviour patterns of the leukocyte in these two diseases are consistent both early and late.

In the literature examined to date there is no record of transition of polycythaemia vera to chronic myelocytic leukaemia in the terms of the metabolic pattern. From this we would believe that the different metabolic capacities of morphologically identical cells are changed by the nature of the disease present.

From the extensive studies made by Valentine et al it is evident that unit cell alkaline phosphatase is increased in a wide diversity of pathological conditions, such as cerebral vascular accidents, myocardial infarction, acute gout, trauma infection, intestinal haemorrhage, haemolytic anaemia, carcinomatosis, reticulosarcoma, myelofibrosis, tuberculosis, stress etc. In almost all individuals whether in health or disease, the stimulation of adrenal activity[d] by the administration of hydrocortisone or A.C.T.H. will raise the normal unit leukocyte alkaline phosphatase 200 to 300 per cent. In disease when the value is already substantially increased, little or no rise may be demonstrated. In the case of true Addison's Disease no increase in urinary 17-Hydroxycorticosteroids is found and no rise in leukocyte alkaline phosphatase is demonstrable when A.C.T.H. alone is administered.

Following the administration of A.C.T.H. a substantial rise in urinary steroid output will demonstrate normal or functioning adrenal tissue, but in chronic myelocytic leukaemia there is very slight or no rise in unit leukocyte alkaline phosphatase. When A.C.T.H. fails to raise the urinary steroid output 17-Hydroxycorticosteroid may be administered. Medication over a three-day period is necessary to show the maximum rise though some increase may be apparent after one day but again in chronic myelocytic leukaemia no response is obtained . (Dosage—A.C.T.H. 40 units

of purified gel 8 hourly for 9 doses—intramuscular. 17-Hydroxy-corticosteroid, 70 mg. and oral hydrocortisone 8 hourly for 9 doses).

It is not unusual in the treatment of chronic myelocytic leukaemia to obtain an apparent clinical and total haematological remission of a temporary nature but it is rare[i] to obtain a return[h] to normal of the unit cell leukocyte alkaline phosphatase even with the assistance of A.C.T.H. or corticosteroid.

Maloney[e] and Lange describe four cases of preclinical myelocytic leukaemia discovered during surveys of atomic bomb survivors in Japan. Slight leukocytosis in the peripheral blood with marked basophilocytosis led to biochemical studies on the peripheral blood leukocytes. The findings included constantly low alkaline phosphatase activity and supported Valentine's suggestion that enzymatic and biochemical abnormalities precede recognisable cytological immaturity. Bone marrow pictures showed no more than a leukaemoid reaction.

METHOD:

The preliminary separation of leukocytes is accomplished by one of the following methods:

(a) Valentine and Beck[h] 1951

15-20 ml. of freshly drawn venous blood is mixed with a freshly prepared saline solution of bovine fibrinogen (Fraction 1), Armour of such concentration that the packed cell volume of the final solution is approximately 30 vols% and its fibrinogen content approximately 7 mg. per ml. Unless it is known that the citrate content of the original fibrinogen preparation is high enough to constitute an adequate anticoagulant, heparin 4 mg. (400 units) per 20 ml. of blood should be added. All foam is removed before allowing to stand for 20-30 minutes when most of the red cells will have sedimented permitting the removal of the supernatant plasma containing leukocytes and platelets largely free from erythrocytes.

(b) Chen and Palmer[k] (1958)

0.2 ml. Heparin (10 mg./ml.) 0.1 ml. of phytohemagglutinin and 1.0 ml. of 6% dextran for each 5 ml. of blood. Invert gently and remove foam and stand for 15-20 mins. Middle supernatant contains the greatest concentration of leukocytes. In polycythaemia dilute the blood to reduce the P.C.V. to approximately 30 Vol.%. (Bactophytohemagglutinin 50 mg. diluted with 5 ml. of special buffer. Difco).

(a) and (b).

Remove the supernatant and centrifuge for 3.0 mins. at 800 r.p.m. Remove plasma and resuspend the cells in saline.

Again centrifuge and resuspend in saline (say 5.0 ml., depending on the leukocyte count) and determine the concentration of leukocytes by standard counts done in quadruplicate. Platelet contamination is largely eliminated by the differential centrifugation.

Reagents	Leukocyte Test	Leukocyte Blank
Sod. B-glycero phosphate	9.0 ml.	9.0 ml.
Saponin	0.5 ml.	0.5 ml.
Magnesium Chloride	0.2 ml.	0.2 ml.
Allow solutions to warm for 3 minutes at 37°C.		
Leukocyte suspension	0.3 ml.	—
Incubate at 37°C. for 60 mins.		
Trichloroacetic Acid	2.0 ml.	2.0 ml.
Leukocyte suspension	—	0.3 ml.
	12.0 ml.	12.0 ml.

Filter through Phosphorus free paper and determine phosphorus by the method of Fiske and Subbarow.

Reagents	Blank	Standard 4·0mg./100ml.	Leukocyte Blank	Leukocyte Test
Trichloroacetic Acid 5%	3.0 ml.	—	—	—
Leukocyte Free Filtrate	—	—	3.0	3.0
Standard P 0.004 mg./ml.	—	3.0	—	—
Ammonium Molybdate 2.5%	0.5	0.5	0.5	0.5
1.2.4. aminonaphthol sulphonic acid	0.5	0.5	0.5	0.5
Distilled Water	4.0	4.0	4.0	4.0
	8.0	8.0	8.0	8.0

Mix and place in dark for 10 minutes. Compare the optical densities of the Blank and Standard and the Sample Blank and Sample Test in the photometer at 650 m.u.

N.B.—(The author has found maximum colour development of the leukocyte phosphorus to take up to one hour although standard or serum phosphorus takes 10 minutes. If this is found to be the case immerse the tubes in boiling water for one minute. Cool before comparing optical densities).

REAGENTS:

Sodium B-glycerophosphate pH 9.9 0.26M.

Dissolve 4.09 g. sodium B-glycerophosphate (315.13) and 2.19 g. sodium barbitone in distilled water by boiling. Cool and make up to 500 ml. Adjust to pH 9.9 by the addition of N/10 NaOH drop by drop. (Only a small amount is needed—probably 0.2 ml.). Keep in refrigerator.

Saponin: Dissolve 2 g. in 100 ml. sterile physiological normal saline. Keep in refrigerator. Remake if moulds appear.

Magnesium Chloride 0.05M: Dissolve 4.76 g. $MgCl_2$ in distilled water, and make up to 100 ml.

Trichloroacetic Acid 30%: Dissolve 30 g. in water and make up to 100 ml.

Fiske & Subbarow Reagents:

Trichloroacetic Acid 5%: Dissolve 25 g. of trichloroacetic acid A.R. crystals in distilled water and make up to 500 ml.

Ammonium Molybdate 2.5%: Dissolve 2.5 g. of ammonium molybdate A.R. in water and make up to 100 ml. This reagent may need to be made up frequently and should not be used if a sediment has formed.

1-amino-2-naphthol-4-sulphonic acid Reducing Reagent: Dissolve by shaking with distilled water and dilute to 100 ml.:—

0.2 g. 1-amino-2-naphthol-4-sulphonic acid.

12.0 g. sodium meta-bisulphite.

2.4 g. sodium sulphite (W/V) crystalline. Filter clear and store in a dark bottle in the refrigerator. This solution should be renewed frequently.

Stock Standard: (10 mg.P per 100 ml.): Dissolve 0.4389 g. of pure dry potassium dihydrogen phosphate (KH_2PO_4) and make up to 1 litre with distilled water.

Working Standard: (0.004 mg./ml.): Dilute 1 ml. of stock up to 25 ml. with 5% trichloroacetic acid to make a working standard equivalent to 4 mg. P per 100 ml.

GENERAL: (Valentine and Beck[b]).

Phosphatase activity given in terms of milligrams of phosphorus liberated per hour by 10^{10} leukocytes is a useful expression, despite the fact that various cell types making up this population may contribute unequally to its total activity. It has been found that leukocyes in health, infection and in myelocytic leukaemia

have characteristic patterns which are most clearly indicated in terms of phosphatase activity per 10^{10} leukocytes.

Normals: From 23 normal individuals.
13.4 - 58.0, mean level 25.8 mg.

Leukocytosis: From 30 persons W.B.C. 11,900 - 32,700.
35.4 - 276.5, mean 119.1 mg.

Chronic Myelocytic Leukaemia: 22 estimations on 14 patients gave 0 - 7.2, mean 4.0. One patient who was suffering from an extensive skin infection gave 14.4 mg.

SUMMARY:

The alkaline phosphatase of leukocytes is discussed.

It is useful as a distinguishing feature of morphologically identical cells.

This examination will demonstrate chronic myelocytic leukaemia before it can be diagnosed clinically or haematologically and it will almost always remain as a persisting stigma in the cases of myelocytic leukaemia in total remission and after steroid therapy.

Finally it distinguishes myelocytic leukaemia from other myeloproliferative disorders, particularly polycythaemia vera and myelofibrosis.

The pituitary-adrenal system appears to control the amount of alkaline phosphatase in the leukocytes although the mechanism of control and the purpose is not clear.

I wish to thank Dr. D. N. Allen, Pathologist, for helpful criticism in the preparation of this paper.

REFERENCES:

(a) Valentine W. N., (1956). Progress in Haematology, **1,** 293.

(b) Wittshaw E. and Maloney W. C., (1955). Blood, **10,** 1120.

(c) Valentine W. N. and Beck W. S., et al (1952). Blood, **7,** 959.

(d) Valentine W. N., Follette J. A., Solomon D. H., and Reynolds J. (1957). The Leukaemias, Henry Lord Hospital, International Symposium.

(e) Maloney W. C. and Lange R. D., (1954). Texas Reports on Biology and Medicine.

(f) Williams M. and Mendel J. L., (1954), ibid.

(g) Quigley H. J., Dawson E. A., et al., (1956). Am. J. Clin. Path. **26,** 109.

(h) Valentine W. N. and Beck W. S., (1951). J. Lab. clin. med., **38,** 39.

(i) Mitus W J., Bergna L. J., et al., (1958). Am. J. Clin. Path., **30,** 285.

(j) Leonard B. J., Israels M. C. and Wilkinson J. F., (1958). Lancet, **1,** 289.

(k) Chen H. P. and Palmer G. K., (1958). Am. J. Clin. Path, **30,** 567.

LIBRARY NOTES

Bela Schick and the World of Children (an authorized biography)
Antoni Gronowicz Abelard-Schuman.

"It is written the power of civilized man." Louis Pasteur had said "to cause the infectious diseases to disappear from the earth."

The older members of the medical profession can remember seeing whole wards full of child diphtheria cases with tracheotomies and yet today this disease occurs only rarely and in civilized countries never reaches epidemic proportions. For this we are indebted to Bela Schick and his associates. Although best known for his work with diphtheria, Schick must also be acclaimed as a great paediatrician. As a pupil of Escherich and associate of Von Pirquet, Schick contributed much to the knowledge we now have of allergies, serum sickness and immunity.

Scientific American January, 1961.

This magazine is well known for its interesting and instructive articles. In this issue are several articles of value to medical laboratories.

1. The Mechanism of Immunity Sir Macfarlane Burnet.

How does an animal make an antibody that neutralizes a specific foreign substance.

2. Glass Charles H. Greene.

Man has made it for 5,000 years, but he is just beginning to learn its nature.

3. The Human Thermostat. T. H. Benzinger.

A newly discovered sensory organ in the brain measures the body temperature.

How long is it since you had fire drill in your laboratory? Are you, whether Charge Bacteriologist or new trainee, alert to fire and earthquake hazards in your place of work? Are fire extinguishers and fire instructions placed in a prominent position and do all the staff members know what to do in case of a fire outbreak? Is your laboratory "housekeeping" beyond reproach?

General Fire Hazards and Fire Prevention.

J. J. Williamson Pitman.

This book has several chapters directly applicable to hospital laboratories. When you have studied these why not ask your local Fire Brigade Superintent for further recommended books.

The above books are all available through Public Libraries or the National Library Service.

"Library Notes" has now appeared in three issues of the Journal. The books so far reviewed have been selected mainly to help trainees in their supplementary reading. The librarian will be most grateful to receive comments and suggestions for future issues.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY (INC.)

Minutes of the meeting held at Wellington Hospital on April 29th, 1961.

Those Present—Mr Olive in the chair; Messrs Hutchings, Donnell, Walker, Bloore, Rose, Cameron, Philip; Misses Mattingly and Scarf.

Mr Olive welcomed the members and the meeting opened at 10.20 a.m.

Minutes of the previous meeting.

Moved:—That they be taken as read. Bloore, Mattingly.

Business arising from the Minutes:

The Secretary reported that the Federal Secretary of the A.I.M.L.T. could not explain the source nor the reason for the information that the N.Z.I.M.L.T. had joined with them in applying for membership of the International A.M.L.T. and that he would write to the I.A.M.L.T. secretary correcting the misunderstanding.

The secretary read the reply received from the N.A.C. It was decided that the contents be explained and discussion invited at the next conference.

The Subcommittee Meeting with the Subcommittee from the Society of Pathologists.

Mr Olive reported on this meeting.

Moved: That thanks be extended to the Secretary and Mrs Hutchings for kindness received on the day of this meeting. Olive, Bloore.

Mr Olive explained that until the Health Department had accepted the report and met on it officially things could not progress further. The Department was expected to meet very shorly.

One of the proposals of this meeting was that the N.Z.I.M.L.T. be asked to explore the assignment system and to bring down a detailed scheme, including an estimate of costs.

Mr Donnell offered to do this.

Moved: That the minutes be confirmed. Donnell, Cameron.

Applications for membership from the following were received.

Miss Pauline Culy, Wellington; Mr B. B. Kirkham, Auckland; Mr M. H. Hampson, Rotorua; Miss M. R. Doran, Tauranga; Miss P. E. Lane, Tauranga; Mr A. J. Forsythe, Dunedin; Miss A. M. Gellatly, Dunedin; Mr A. J. Gray, Dunedin; Mr G. N. Tannock, Dunedin; Miss E. L. Scott, Dunedin; Mr A. G. Neilson, Dunedin; Mr E. K. Fletcher, Dunedin; Mr B. F. Pidd, Kaitaia; Miss H. M. Bond, Wellington; Mr D. J. Pitches, Nelson; Mr D. A. MacDuff, Ashburton; Miss A. D. Paykel, Auckland; Mr J. G. Whitefield, Kaitaia; Mr B. T. Edwards, Christchurch; Mr M. J. Grattan, Christchurch; Mr A. R. Coates, Christchurch; Mr I. R. Orchard, Christchurch; Miss D. M. Moylan, Christchurch; Miss H. M. Hannah, Christchurch; Miss J. B. Speden, Christchurch.

Moved: That the applications be approved. Donnell, Mattingly.

Resignations: The following resignations were received.

Mr H. Ward, Timaru; Mrs C. Cartwright (nee Curtis); Mrs J. A. Hough, Christchurch; Miss S. Sinclair, Christchurch; Miss J. D. Clarke, New Plymouth.

Moved: That the resignations be accepted with regret and that the unfinancial members be deemed resigned. Donnell, Mattingly.

Treasurer's Report: The Treasurer reported that there were 204 Junior and 148 Senior Members. He also reported that with the payment of subscriptions in arrears the finance of the Institute was satisfactory.

Moved: That the Treasurers report be accepted. Philip, Bloore.

The Treasurer pointed out that in the future only an increase in subscriptions would maintain an excess of income over expenditure.

Moved: That the change of name at the Bank of N.Z. be authorised and the cheque signatures be Messrs D. Philip, G. Cameron and M. Donnell. Philip, Bloore.

Journal Report: The Editor reported that the next issue of the Journal was in the hands of the Printer.

Moved: That the Frontpiece block of the Journal be changed to be in keeping with the change of name of the Institute. Rose, Cameron.

Correspondence: A copy of the rules of the Dunedin Branch of the N.Z.I.M.L.T. was tabled and perused. The request for approval of this branch was read.

Moved: That the Dunedin Branch of the N.Z.I.M.L.T. be recognised and that the Secretary write wishing them well. Donnell, Walker.

Moved: That the Secretary on behalf of the N.Z.I.M.L.T. (Inc.) make application for membership of the Public Service Investment Society Ltd. Donnell, Mattingly.

Rex Aitken Memorial Prize.

Moved: That Mr G. Rose be nominated as the Institute representative on the panel of Judges for the Rex Aitken Memorial Prize. Donnell, Mattingly.

Moved: That the Secretary write asking Dr. Stewart of Christchurch to act with Mr Rose and a Director of Biological Laboratories Auckland. in Judging a winner of the Rex Aitken Memorial Prize. Cameron, Scarf.

Moved: That a review of appointments of all existing subcommittees be made at the next council meeting. Donnell, Walker.

Moved: That inward correspondence be received and outward correspondence be approved. Olive, Donnell.

General Business.

Salary Submissions:

Moved: That the Secretary write to the Secretary of the N.Z. Association of Scientists requesting information concerning the current investigation to the salares of N.Z. Scientists and enquiring as to whether the N.Z.I.M.L.T. could be of any assistance in this enquiry. Bloore, Mattingly.

Some discussion followed on salaries and submissions. It was finally decided to bring the matter to the agenda of the next council meeting.

Moved: That the following expenses for this meeting be paid by the Treasurer. Mattingly, Olive.

Travelling expenses for the following:—	£	s.	d.
Mr H. Bloore	3	18	6
Mr H. Hutchings	1	7	6
Messrs Donnell, Cameron and Philip	39	1	6
Miss Scarf	5	10	0
Mr Rose	9	0	0
Mr Walker	9	9	9
Luncheon expenses of	1	0	0
	£68	17	6

Mr Donnell moved a vote of thanks to the chair and the meeting closed at 4.40 p.m.

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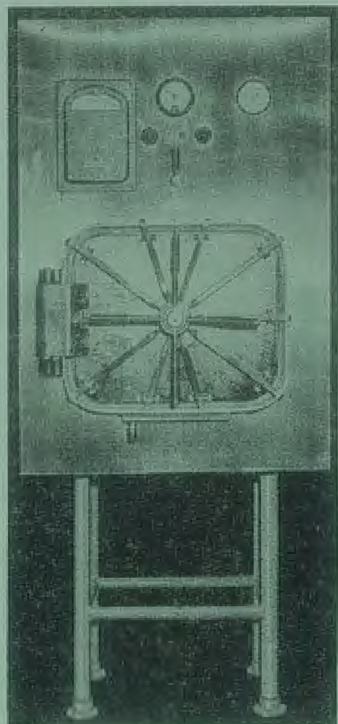

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