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# JOURNAL

## OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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# HAEMOGLOBIN AND ITS ESTIMATION—A REVIEW

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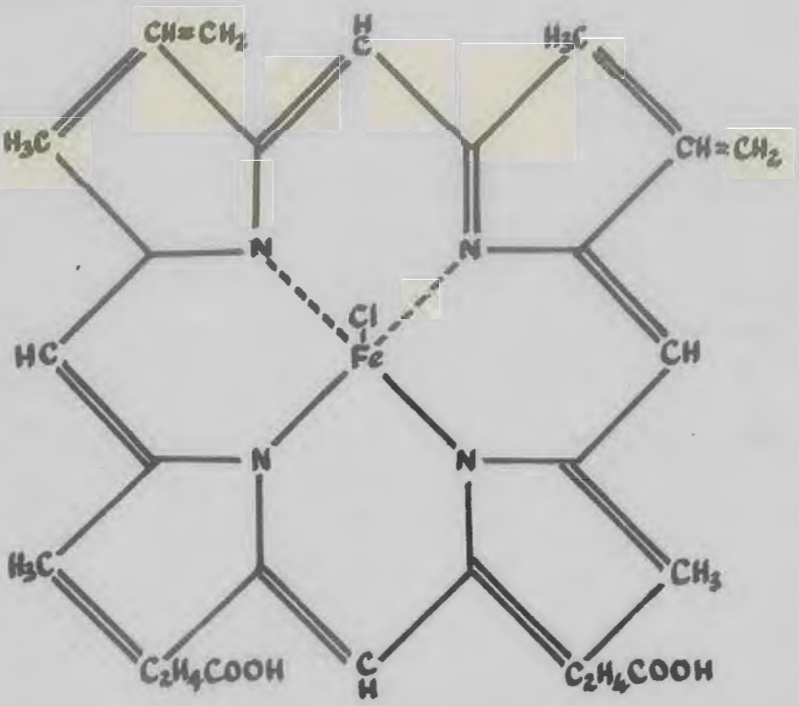
Haemoglobin has been an important estimation from the clinical viewpoint for nearly 75 years and in lieu of this history it is astonishing to find the insouciant attitude which the average laboratory attaches to this estimation. Throughout the world diverse techniques are used and even within more limited spheres a wide range of preferences are found. It must be admitted that provided the results forwarded by one institution are consistent, then from the clinical viewpoint the technique is satisfactory. However, this attitude can hardly be condoned from a technical aspect and moves, which are being made to abolish inconsistencies such as differences in methodology and the lack of an accepted standard for haemoglobin, are to be encouraged. Primary observations on haemoglobin date back to Robert Boyle, who in 1683 first considered the possibility that blood served a respiratory function and speculations on the role of the red pigment in the blood were recorded in his memoirs.

The first serious studies upon crystals from blood were undertaken by Virchow, who assumed that blood crystals were composed of a blood pigment haematin in combination with a protein. Assuming that the combination of these elements resulted in the formation of crystals he treated the latter with nitric acid. Because the xanthroproteic reaction which resulted was similar to that obtained with albuminous substances he assumed that the protein concerned here was the same. In 1862 haemoglobin was isolated in crystalline form by Hope Seglar. Gowers (1878) is associated with the development of the first apparatus for estimating haemoglobin. His apparatus consisted of a small stand that held two glasses of equal bore. One tube contained a coloured standard compound of a mixture of glycerine gelatine carmine and picrocarmine. The other tube was calibrated and dilutions of of blood were made in water until the colour matched that of the standard. Haldane and Smith were the first to devise a method for measuring the oxygen capacity of blood. A method which is still contemporary.

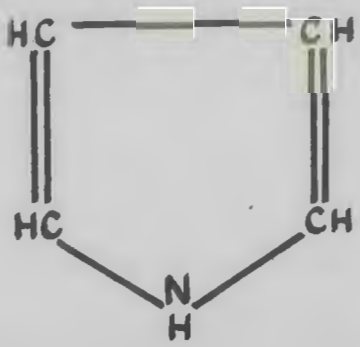
## *CHEMISTRY OF HAEMOGLOBIN*

Haemoglobin is a readily crystallizable conjugated protein consisting of a colourless protein portion, globin and a coloured non-protein portion, the prosthetic portion, which is known to be an iron containing portion belonging to the class of porphyrins. The iron containing portion is known as heme or haematin. The chloride of heme (unreduced form) haemin has been known for many years and may be considered as a chloride compound of

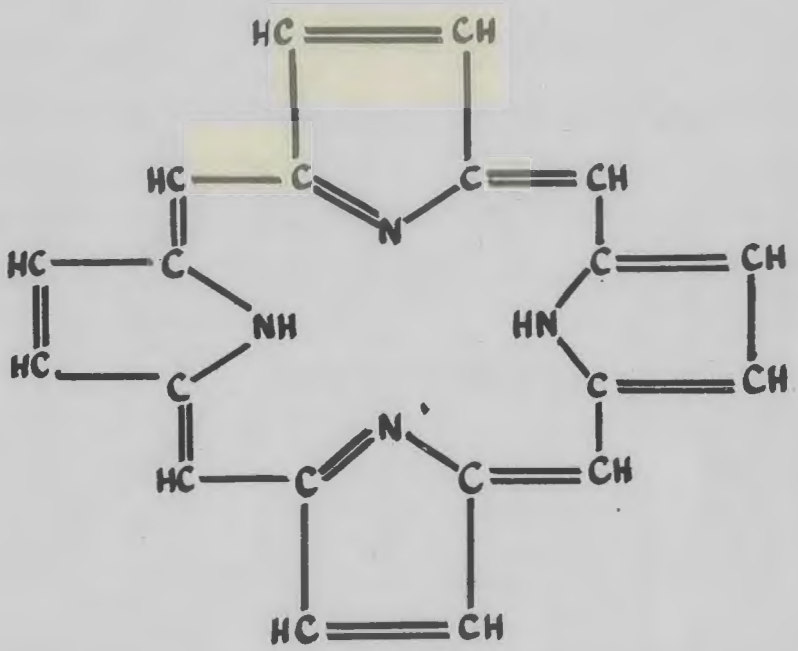
heme. Haemin has been synthesized and its structure has been represented thus:




Both are iron porphyrin combinations. Heme itself is derived from an iron free compound known as protoporphyrin IX and together with the globin forms haemoglobin. It can be seen that the haemin molecule contains iron in the trivalent form and the rest of the molecule consists essentially of four substituted methyl pyrrole rings joined together by —CH = bridges. The chief structural unit of protoporphyrin is the pyrrole ring



Four pyrrole rings are joined by four methane bridges to form the larger ring porphin, which is the structural precursor of all porphyrins




The porphyrin of haemoglobin and heme compounds is protoporphyrin. Pauling showed that the chemical characteristics of haemoglobin result from the magnetic and electrical interaction of the iron and porphyrin ring, which prevents the iron from forming further bonds with most other compounds, except oxygen and carbon monoxide. The combination of haemoglobin with oxygen and carbon monoxide has a profound effect on the electronic properties of heme and globin. The changes are detected in the change in spectrum and in magnetic moment. There are numerous physical and chemical properties of haemoglobin, many of which have been used as a basis for estimation of this compound. These estimations have largely depended on the ability of haemoglobin to react with oxygen, carbon monoxide, nitric acid, hydrogen sulphide and ferricyanide, the product in most cases being a hemochromagen. The latter may be identified qualitatively by spectroscopic examination and quantitatively by use of the spectrophotometer.

## *THE HAEMOGLOBIN STANDARD*

A basic necessity of any quantitative method is the provision of a standard in terms of which the results can be expressed. Haemoglobinometry is no exception and in its early days direct comparison of results obtained by various techniques were impossible when each original contributor adopted an arbitrary colour standard. 100% of the Haldane scale was equivalent to 107% on the Dare, 93% on the Tallqvist, 97% on the Gowers and 86% on the Sahli instruments. From 1900 to 1930 Haemoglobinometers were standardized by gasometric determinations and in many cases were calibrated to read in grams of haemoglobin per cent. This lead to more confusion as using the most precise methods of determination the colour-oxygen capacity of normal haemoglobin samples may vary by ± 7% due to inactive haemoglobin. Donaldson, Harding and Wright (1943) contributed an advance in the establishment of a haemoglobin standard by establishing a carboxyhaemoglobin standard at the National Physics Laboratory (N.P.L.). The N.P.L. standard though practically permanent is defined in trichromatic terms so that the colour can be reproduced at any time. With this fixed point firmly established the next step was the determination of the haemoglobin concentration equivalent to the standard colour. This was undertaken by McFarlane et al 1944. The basis of their standardization programme was the colorimetric comparison of a series of blood samples with the N.P.L. standard by means of the colour comparator and spectrophotometer in parallel with gasometric and iron determinations. The large number of samples used and the care taken to eliminate all sources of error make it probable that the results obtained are reliable. It was found that if the haemoglobin mass is obtained from oxygen capacity the Haldane standard is equivalent to 14.4g. of haemoglobin and to 14.8g. if mass is assessed from iron determinations. The lower figure is almost certainly due to the existence of haemoglobin or its derivatives which do not take up oxygen. The higher figure is likely to represent the true haemoglobin mass and has been taken as such. The establishment and definition of the N.P.L. standard haemoglobin now allows all instruments employed in clinical haemoglobinometry to be calibrated on an equal basis.

## *ESTIMATION OF HAEMOGLOBIN*

The large number of methods which have been suggested for the estimation of haemoglobin are evidence of the real difficulty attending this estimation. The following is an account of the more important procedures with a short comment on their suitability. The Tallqvist and copper sulphate methods are included more

for their historical interest than their usability. The haemoglobin content of a solution may be estimated for standard or reference purposes by

(a) Measurement of its colour.

(b) By its power of combining with oxygen or carbon monoxide.

(c) By its iron capacity.

The colour matching techniques described measure, together with the specific pigment for which the test is designed, inert pigments such as methaemoglobin and sulphaemorglobin, which may be present. Ideally as a functional estimation of haemoglobin, measurement of oxygen capacity should be carried out. This is not practical in clinical work. Iron content of haemoglobin can be estimated accurately but again the method is impracticable for clinical purposes. For routine purposes colorimetric procedures for the determination of haemoglobin are preferred to the more laborious techniques. However, they suffer the disadvantage that frequent standardization must be conducted by measurements of the iron content of the blood to guard against possible deterioration of the standard. Difficulties have been encountered in the choice of standards for use in colorimetric purposes, not only on the score of unsuitability, but also because the absorption spectra of the artificial standards hitherto proposed have usually been widely different from those of the blood derivatives they intend to match.

## *ALKALINE HAEMATIN METHOD USING GIBSON-HARRISON STANDARD*

To overcome these difficulties Gibson and Harrison developed an artificial standard consisting of a mixture in aqueous solution of chromium potassium sulphate and cobaltous sulphate. The preparation is stable over a considerable range of wavelengths and reproduces closely the absorption spectrum of blood treated with dilute alkalis.

### *Method*

0.05 ml. of blood is added to 4.95 ml. of N/10 NaOH and heated in a boiling water bath for 3 minutes. It is then cooled rapidly in cold water and matched against the standard in a photoelectric colorimeter using a yellow green filter. The Gibson-Harrison standard has been adjusted to equal 15.6 g. of haemoglobin by oxygen capacity and 16.0 g. per 100 ml. by iron content of blood. It is essential to heat the standard along with the test sample. Only after heating which alters the ionization of the salts it contains does the ability of the standard to absorb green light approximate closely to that of alkaline haematin.

*Comment*

This is a useful ancillary method as it gives a true estimate of total haemoglobin even if carboxyhaemoglobin, methaemoglobin or sulphaemoglobin are present. A true solution is obtained and the plasma proteins and lipoids have little effect on the development of colour if the blood and alkali are thoroughly mixed. A disadvantage of the method is that foetal haemoglobin is resistant to alkali denaturation, but this can be overcome by heating the solution in a boiling water bath for 5 minutes. In normal circumstances the method is more cumbersome and less accurate than oxyhaemoglobin.

## *ACID HAEMATIN METHOD (SAHLI)*

*Method*

The graduated tube is filled to the 20 mark with N/10 HCl and 0.02 ml. of blood are mixed in. After 5 minutes the brown solution is diluted with N/10 HCl until the colour of the solution matches that of the standard. The level of the haemoglobin is read directly as a percentage or in grams from the calibrations on the calibrated tube. The best type of apparatus has two standards between which the graduated tube is placed. The value of the standard varies with the maker and may be between 13 and 17 grams per 100 ml. It should always be checked by the approved methods.

*Comment*

This popular method which is used by small institutions and by general practitioners has serious disadvantages. The standards which are usually made of brown glass vary in their haemoglobin equivalent and may fade. They usually need recalibrating. The brown colour of acid haematin takes time to develop to its greatest intensity depending on the temperature, protein and lipoid content. Acid haematin is present in colloidal form rather than true solution and this may result in turbidity which makes colour matching difficult.

## *CARBOXYHAEMOGLOBIN*

Blood is diluted in a solution of ammonium hydroxide which results in it being laked and then saturated with carbon monoxide. The concentration of carboxyhaemoglobin is measured in a photometer or spectrophotometer. 10 ml. of 0.4% ammonium hydroxide is placed in a test tube and 0.05 ml. of well mixed blood added. Carbon monoxide is bubbled through this mixture for 30 seconds and the optical density read in an instrument calibrated as will be described for oxyhaemoglobin.

*Comment*

The carboxyhaemoglobin methods lack the simplicity of the oxyhaemoglobin methods. They provide greater absolute accuracy, however, not only because of the greater stability of the solutions but also because any carboxyhaemoglobin present in the blood is measured along with oxyhaemoglobin and reduced haemoglobin.

## *OXYHAEMOGLOBIN*

In 1929 Sheard and Sanford presented the first photoelectric method for measuring haemoglobin. Their method consisted of converting haemoglobin to oxyhaemoglobin by shaking with an excess of a dilute solution of sodium carbonate. However, it was noted that the oxyhaemoglobin solution tended to fade because of the relatively high temperature, high dilutions and high pH of the solution. Subsequent modifications of this method were directed towards the reduction or elimination of fading of the oxyhaemoglobin solutions and to achieve this objective numerous diluents were used. The best results were obtained with 0.04% ammonium hydroxide. The oxyhaemoglobin methods for determining the haemoglobin content of the blood are simple, rapid and accurate. One possible disadvantage of the method is its failure to measure other haemoglobin derivatives such as carboxyhaemoglobin which may reach high proportions in some cases.

*Method*

Oxalated blood is serially diluted with physiological normal salt solution. On each sample simultaneous determinations are made of the iron concentration and the readings of the colorimeter of choice by the technique described below. The iron concentration is changed into its haemoglobin equivalent. A chart is prepared plotting the haemoglobin values against the readings on the colorimeter by the method of choice, which has been performed in conjunction with the iron determinations.

*Routine Used*

8 ml. of 0.04% ammonium hydroxide is placed in an optically suitable test tube and 0.02 ml. of blood added. Rinse pipette, stopper tube and shake vigorously for 10 seconds. Read in the instrument which was used in the original calibration at wavelength 545 mu.

*Calculations*

Read the haemoglobin value direct from the calibration curve prepared from the haemoglobin measurement by iron estimations.

## *HAEMOGLOBIN ESTIMATION BY IRON CONTENT (WONG).*

Iron is detached from the haemoglobin and other protein molecules by the action of concentrated sulphuric acid in the presence of potassium persulphate without heating. The proteins are then precipitated by tungstic acid and the iron is determined colorimetrically using the filtrate by the thiocyanate reaction. Since the process involves no boiling the acid concentration in the unknown can be controlled and duplicated in the standard for the production of colour. As most of the iron present in the blood is in the form of haemoglobin the iron concentration closely approximates the haemoglobin content. The haemoglobin concentration may be obtained following the iron estimations from the following equation $\frac{\text{mgs. iron}}{3.4}$ per 100 ml. = grams haemoglobin per 100 ml.

Compared with the difficulties of the estimation of haemoglobin from the red colour of the blood and allied techniques the various methods for the estimation of iron are for the most part concise. The methods are accurate to within ± 1% and although they are too time consuming to use routinely they are useful for the calibration and checking of the standard methods used daily.

## *GREY WEDGE PHOTOMETER USING GREEN FILTER*

It has been shown that a neutral-grey screen can be used conveniently as a standard in the Duboscq type of colorimeter for any coloured solution if an appropriate light filter is interposed. The instrument consists essentially of an annular grey wedge mounted in a glass disc which can be rotated so that more or less light is transmitted by the segment viewed by the observer. A diffusing screen allows light to pass either through this segment of the wedge or through the cell containing the haemoglobin solution. The two light beams are then brought together by a prism so that the observer sees the two fields as adjacent halves of a circle. The appropriate green filter is mounted in the eye piece where it intercepts both light beams. The brightness of one half of the field can be varied to match the other by rotating the wedge which carries a scale on its rim, so calibrated that the 100% reading with the specific filter is equivalent to the haemoglobin concentration of the N.P.L. Haldane standard. Errors for the method are in the vicinity of 5%.

### *Method*

0.02 ml. of blood is washed into 4 ml. N/150 $NH_4OH$. After mixing by inverting several times the solution of oxyhaemo-

globin is ready for matching in the photometer. The density of light transmitted by the test solution is matched in an adjacent half field against the light transmitted by the rotating grey wedge. The haemoglobin is read off as a percentage (Haldane scale) 100% = 14.8 g. haemoglobin.

## *ESTIMATION OF HAEMOGLOBIN BASED ON THE COLOUR OF HAEMOGLOBIN*

The depth of red colour of the blood is directly proportional to the concentrates of iron and the haemoglobin present. The red colour therefore may be compared with that of various preparations used as standards and the concentration of haemoglobin determined. Tallqvist (1900) devised a simple method of transferring a drop of blood to a piece of filter paper and comparing the colour with a series of lithographed standard colours. Later this technique was modified and the Tallqvist colour scale was transferred to a circular disk which was enclosed in a watch like case with a small window so that the scale could be turned to any desired portion for comparison with the unknown blood sample. Both methods have the common difficulty in matching various shades of red. Errors may range from ± 10 to 40%.

## *SPECIFIC GRAVITY METHODS*

The specific gravity of whole blood is related to the concentration of its constituents of which haemoglobin is the most important by weight. Routine methods of estimation depend on the rising or sinking of drops of blood or plasma in fluids of known specific gravity, such as the solvent mixture column of the Linderstrom Lang method or the range of copper sulphate solutions of the Van Slyke system. Both the Tallqvist and the above method are within the limits of accuracy required for such screening tests as the selection of blood donors.

## *REFERENCES*

Dacie, J. V. (1950), "Practical Haematology" London: Churchill.

Dyke, S. C. (1951), "Recent Advances in Clinical Pathology", 2nd ed. London: Churchill.

Hawk, P. B., Oser, B. L., Summerton, W. H. (1947), "Practical Physiological Chemistry", 12th ed., London: Churchill.

Sunderman, F. W. et al. (1953) Am. J. Clin. Path., *23,* 519-520.

# THE DEVELOPMENT OF THE MODERN MICROSCOPE

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(*Pathology Department, Wellington Hospital*)

Winner Junior Essay Competition, 1960.

Of all th' Inuentions none there is Surpasses,
The nobel Florentine's Dioptrick-glasses,
For what a better, fitter, guift could bee,
In this world's Aged Luciocity,
To Helpe our Blindness so as to deuize,
A pair of new and Artificiall eyes,
By whose augmenting power wee now see more,
Then all the world Has euer donn Before.

Dr. Henry Power 1661.

## *INTRODUCTION*

Who invented the microscope? Zaccharias Janssen, of Holland, Galileo, of Florence, and Robert Hooke, of England, have all been accredited with the invention of the microscope. Some say that Janssen, while experimenting with a telescope found it could be adapted for microscopical work, others that Galileo placed two lenses in a tube, which he mounted for focusing and then announced his discovery of the microscope in 1610; the Royal Microscopical Society award the honour to Robert Hooke. It is, however, doubtful if the invention of the microscope could be attributed to any one man, as developments leading to the eventual construction of the first microscope had been started many years before the time of Janssen, Galileo and Hooke, and improvements since the time of these men have made microscopes of their era not only utterly obsolete but almost unrecognisable.

## *OPTICAL SYSTEM*

The optical system of the microscope consists of the objective, the eyepiece, and the condenser. The essential part of these three components is the lens, so that the development of the lenses and a proper comprehension of their properties was needed before the simplest of microscopes could be constructed and it was of paramount importance to correct their aberrations before the efficient instruments of to-day could evolve. The word lens comes from lenticula-lentil—a bean-like vegetable with seeds resembling double convex lenses, and Pliny the Elder spoke of "the burning properties of lenses made of glass". Earlier still, Seneca, the Roman philosopher who died 65 A.D., spoke of a glass globule filled with water which "will aid in seeing those difficult things that frequently escape the eye". The first practical use of lenses was in spectacles, their invention usually being accredited to D'Armato.

The English, on the other hand, attribute the invention of spectacles to Roger Bacon, who lived 50 years before D'Armato. More than two hundred years passed before we find the first reference to the modern microscope; the telescope. This was in 1542 when Nicholas Copernicus of Poland, using a crude instrument of his own design, observed stars and planets. We come now to the early part of the seventeenth century, when the microscope first made its appearance and it was in 1611 that the first mathematical exposition of the theory of optics was made, although unsuccessful attempts had been made earlier by Ptolemy in the 2nd century; Alhazen in the 11th century and Roger Bacon in the 13th century. This exposition was made by Johannes Kepler of Germany who also constructed a microscope using convex lenses throughout, thus obtaining an inverted image of the object under observation. During this period a discovery of the utmost importance to optics occurred. This was Campini's discovery that lenses could be ground. Previously lenses had been formed on the ends of glass rods by heating, but naturally it was not possible to reproduce any given curvature. Many substances have been tried for lenses. Among the more unusual were the attempts of Wilson Marshall and Adams with different types of varnishes, which were unsuccessful due to their rapid oxidation; and Andrew Pritchard's efforts with gem stones which were also a failure because of the natural colouring of these stones.

The initial magnification of an object under observation, is produced by the objective of the microscope. However, when an objective if magnified by a simple lens, a true image of the object is not obtained due to (a) curvature of the image; (b) astigmatic; (c) spherical and chromatic aberration. Thus the development of the objective has been carried out mainly in correcting these faults. Newton experimented with different glasses and other media and concluded that the dispersive power was directly proportional to the refractive power, which meant that it was impossible to correct the aberration of lenses. Fortunately, Newton, for once, was wrong as was discovered by an amateur English optician Charles Hall, when he found that flint glass, which has almost the same refraction power as crown glass, has approximately double its dispersive power. Hall's idea was pursued by John Dolland who by experimenting with combinations of "hard" and "soft" glass, put on the market in 1758 an achromatic system of lenses although these were for use in a telescope. Joseph Fraunhope constructed an achromatic objective, reportedly the first, but this objective was not credited with being of value by his contemporaries. However, Harmanus van Deijl of Amsterdam did have achromatic objectives on the market by 1807. In 1824 the French physicist

Sellique found that by screwing several low power achromatic lens systems together, it was possible to achieve sufficient magnification without grinding lenses with extremely short focal lengths. It did not take long for it to be realised that the individual systems of lenses need not be achromatic as long as their respective errors were mutually compensated. Two men who became interested in these developments were Lister of England, who made further improvements, and Professor Amici of Italy. Amici, who had tried and failed to produce an achromatic objective previously, renewed his efforts and constructed an objective with three sets of adjustable lenses; this type of objective was adopted by most microscope makers for many years. At the same time in England, Tully, working independently, also developed an achromatic triplet which, although of low power, was better corrected than any previous or contemporary objective. A period of intense competition now began, to see who could make the best corrected and highest powered achromatic objective. Thus objectives were made with focal lengths of 1/50, 1/75 and 1/100 of an inch. By 1851 Charles Spencer in the U.S.A. was reputedly making the best objectives in the world. He decided, after much experimenting, that fluorite was the best substance for a lens, and produced a fluorite objective that was corrected in many ways in a manner similar to today's apochromatic objectives. It was up to Carl Zeiss and his partner Ernest Abbe, however, to make the true apochromatic objectives. Before Zeiss, opticians made hundreds of simple lenses and with patient grinding were fortunate if they produced one or two satisfactory objectives. It was Zeiss who had enough of "dem ewigen bei uns Optikern gebrauchlichen Probieren," and spent his time in calculating the correct radius, thickness, and curvature and turned out a series of precision lenses from his calculations. With these lenses Zeiss and Abbe were able to manufacture, in 1886, an apochromatic objective, i.e. an objective corrected for three different colour wavelengths and for spherical aberration, whereas the achromatic objective was corrected for two chromatic aberrations only. Abbe also perfected the immersion objective. The fineness of detail which an objective is capable of revealing is directly proportional to its numerical aperture. The numerical aperture is found by the formula N sin U, where U is half the angle of aperture of the objective and N is the refractive index of the medium between the object and the objective. Hence the nearer N is to the refractive index of glass, the more detail it is possible to see with the objective. Hooke first noticed this phenomenon in the seventeenth century when he placed water between the object and the objective and in the late eighteenth century Dr. David Brewster experimented with immersion lenses but his work was not continued.

The first systematic application of immersion fluids was made by Amici around 1850 using water, and later glycerine and oils. In America, Tolles, who had worked for many years with Spencer branched out alone and manufactured an objective using balsam as the immersion medium. Then in 1878 Abbe and Zeiss produced the homogenous oil immersion lens, for use with cedar oil, which has a refractive index of 1:51 compared with glass around 1:50. An important contribution to the development of the objective was made by Ross of England, when in 1837 he found the principle of cover-glass correction and made objectives with correction collars. With encouragement from Smith in 1854, Ross also produced a quartz objective for the utilization of light of short wave lengths. This was followed up by Kopler of Germany in 1906, and later by Barnard and Beck in England.

Eyepieces, or oculars, are essential in the optical system of the microscope, as they magnify the already magnified image produced by the objective, giving either real or virtual images. There can also be calibrations incorporated in them for micro-metric work. Eyepieces were brought to a high standard in the seventeenth century, and since then there has been very little improvement on them. In 1690 Christiaan Huygen laid down the principle for the position and curvature of the lenses in the eyepiece and eyepieces based on these principles and known as Huygen eyepieces are still in use today. It is worthwhile to note that the field lens, although part of the objective, is usually incorporated in the eyepiece.

There are, however, eyepieces of different design to that of Huygens which are used mainly for special purposes. The most notable example is the Ramsden eyepiece which was produced by Jesse Ramsden in 1793 and is used for micro-metric work. This eyepiece produces a real image beneath the field lens, whereas a Huygen eyepiece produces a virtual image between the field lens and the eye-glass. A more recent innovation is Watson's Holoscopic eyepiece. As apochromatic objectives are over-corrected, compensating eyepieces are needed for each set of apochromatic lenses. The holoscopic eyepiece has built into it a draw-tube, which dispenses with the need for compensating eyepieces.

Wredden writes that "the microscope used without a condenser is nothing more or less than a magnifying glass, and the quality of the condenser is important if a good performance is to be obtained from a first-class objective. As early as 1691, Buonanni and three years later, Hartsoeker, fitted condensers to their microscopes, but only as a means of intensifying the light. It was not until 1892 that the condenser began to be used seriously, and then only in Britain. It was in that year that a lens system known as Wollaston's Doublet was used as a condenser and Wollaston initiated the art of focusing the condenser. Pritchard

improved on the Wollaston Doublet by placing a diaphragm between the lenses.

The necessity of correcting the condenser began as soon as the use of corrected objectives came into general use and we find David Brewster stating, in 1831, "I have no hesitation in saying that the apparatus for illumination requires to be as perfect as the apparatus for vision, and on this account I would recommend that the illuminating lens be perfectly free from chromatic and spherical abberation". The use of corrected condensers was, for many years, employed in Britain alone, and by 1865 Powell and Lealand were producing a good achromatic condenser with an angle of 170 degrees with air which was in fact the forerunner of the modern corrected condenser. Powel and Lealand also produced the first chromatic condenser for use in an immersion system, but soon afterwards changed to an achromatic condenser for the immersion system. A condenser still popular today is the Abbe chromatic condenser. This condenser was produced by Abbe in the latter part of the nineteenth century and although it is still an admirable for routine work and students' use, it did set microscopical technique back by a number of years as it is in no way corrected, and serves only to concentrate the light. Abbe's condenser received high acclaim when it was first brought out, but the praise came from workers who were accustomed to microscopes with no condenser at all. British supremacy in condenser production was again proven in 1901, when Watson and Sons produced the "Holoscopic" oil immersion condenser. This has an apperture of 1.33 and the only improvement in today's condensers is the enlargement of the aperture to 1.37.

*ILLUMINATION*

"The microscope should be considered to begin at the source of light which is used to illuminate the specimen", state Munoz and Charipper. There are two methods of illuminating the specimen, reflected light and transmitted light.

The oldest drawing of a microscope is that of one made in 1631, which shows the use of reflected light as the illuminate. Descartes first used a concave mirror to focus light on the object in 1637 and this was placed in practical use a century later by Lieberkuhn. Robert Hooke in 1665 used a bi-convex lens to focus light from a small oil lamp on to his object. These two methods were used up to the nineteenth century. John Cuff being the first to apply the Descartes-Lieberkuhn mirror to the compound microscope. During the nineteenth century, preference was given to a type of bi-convex lens known as the "bulls-eye" due to the fact that the concave mirror had to be placed very close to the object and was often shaded from the source of light.

However, during this period, the use of the microtome became widespread, and with the cutting of transparent sections the use of reflected light gave way to the general use of transmitted light and today reflected light is only used in dissecting microscopes.

Leeuwenhoek was the first person to make ample use of transmitted light. In the daytime he held his simple microscope to the sunlight, and at night-time used a candle with a concave mirror behind it to intensify its light. Huygen in 1678 fitted a candle and condenser in a tube, and mounted a small simple microscope on a collapsible optical bench. In 1694 Hartsoeker improved on Huygen's microscope by constructing a small microscope consisting of three cylinders that screwed into each other. The widest cylinder contained a magnifying lens, the middle one served for focusing and the narrowest cylinder contained a condenser. This type of hand microscope was used by amateur microscopists for many years and was known as the Wilson Microscope. The Italian Buonanni and the Englishman Marshall adapted Huygen's method of illumination to compound microscopes but their arrangements were too cumbersome to be of practical use.

The use of a sub-stage mirror was first mooted by Constantijn Huygen in 1679, but the idea was not put into effect until 1716 by the German physicist Hertel. The Englishman Culpeper modified the system by replacing Hertel's plane mirror with a concave one which acted also as a crude condenser.

The first sources of light used were daylight and candlelight. The beginning of the nineteenth century saw the advent of the "solar microscope". This microscope was set up in a dark room with the mirror extended outside to capture the direct rays from the sun and was used extensively for micro-projection. The next step was Drummond's use of limelight which was followed in 1845 by the electric carbon-light.

When transmitted, light is used in an oblique manner, dark ground illumination is produced. In this method the rays of light are transmitted from the condenser in such an angle that only the rays reflected by the organisms under observation enter the objective so that the organisms appear as sources of light in a dark background. The resolving power of the objective, as was shown by Abbe, can be almost doubled by using this technique. This phenomenon was known to the early microscopists Leeuwenhoek, Hooke and Huygens and was first applied during the first half of the nineteenth century. As the rays of light must be oblique a special condenser must be used. In 1852 Wenham used a parabolic mirror which he later modified into a solid piece of glass with a parabolic surface reflecting the light. The light can be obtained even more obliquely with the use of the cardioid condenser mar-

keted by Zeiss in 1908. The use of the cardioid condenser is known as ultra-microscopy.

Two other types of illumination are obtained with the use of polarised light and monochromatic light. Polarised light was pioneered by Talbot in 1834 and is invaluable in research. Monochromatic light, although it does away with chromatic abberation, is of little value in medical science as it makes histological staining meaningless.

*SPECIAL TYPES*

Until now, we have dealt mainly with the monocular compound microscope. Three other types of microscope which deserve mention here are the simple microscope, which was popular during the eighteenth century; the binocular compound microscope, which is used extensively today; and the electron microscope, which is of great value in research work.

The simple microscope came into use soon after the invention of the compound microscope, its most illustrious user being Leeuwenhoek. Until 1830 the simple microscope gave more accurate results than the compound microscope due to its being free from chromatic abberations. However, with the achromatization of objectives, the compound microscope became the better instrument and the simple microscope faded from the scene until today its only use is for dissecting.

The binocular microscope depends on the use of prisms to divide the light into two paths. The early microscope, e.g., Cherubin's in 1677 and Zahn's in 1702, which had two eyepieces, did not use prisms, but had two tubes and were virtually two microscopes focused on the same point. One of the first to use a prismatic system was Riddell in 1853 and with his system, known as pseudoscopic, the light from the left side of the object enters the left eye and vice versa. Wenham in 1860 reversed this so that light from the left side of the object enters the right eye and is known as orthoscopic. The system used today is that produced in 1913 by Jentzsch whereas light from both sides of the object enters the left eye and the right eye.

The most modern development in microscopy is the electron microscope.

The electron microscope can be said to be analogous to the optical microscope. In it a flow of electrons is used rather than rays of light and the "lenses" are a magnetic field. With the electron microscope the image must be either projected on to a screen or photographed. As air interferes with the flow of electrons the whole microscope must be in a vacuum. However, despite these problems, the electron microscope offers immense possibilities in the field of microscopy.

## *AID TO MEDICAL SCIENCE*

Few instruments have been developed which have benefited medical science more than the microscope. From 1660 to 1723 is known as the period of the classical microscopists: Marcello Malpighi, Robert Hooke, Jan Swammerdam, Reinier de Graaf, and the most famous of all Antoni van Leeuwenhoek who is the "Father of microbiology". The more important discoveries of this period include those of the blood capillaries, erythrocytes, mammalian ovian follicle, protozoa, bacteria in infusions and sputum, yeast cells, and leukocytes.. It was during this period that Hooke gave science the term cell by observing "cells" in fossil wood.

For a century after Leeuwenhoek's death in 1723 very little progress in microscopy was made. However, during this time the microscope was developed into an efficient instrument and the way was paved for the startling discoveries that were made from 1830.

1830 to 1878 saw the reaping of the benefits derived from the achromatization of objectives. It was between these years that the world's two most noted bacteriologists began their investigations and the works of Pasteur and Koch retain their importance in medical science. Important among the many discoveries of this period were those of the causative agent of favus, embryonic character of cancerous tissue, lactic acid bacterium, Trichinellae, Entamoeba coli and Entamoeba histolytica. This period saw the publication of the general cell theory; the establishment of histology as a separate branch of science; the refutation of the theory of spontaneous generation by Pasteur; the practice of antiseptic surgery by Lister; Koch's description of the complete life cycle of the anthrax bacillus.

From 1878 the technique of homogenous immersion was applied to microscopy and a rapid discovery of pathogenic micro organisms followed, of which the more noteworthy were:—The cocci organisms including Gonococci, Diplococci, Streptococci, Staphylococci and Pneumonococci; Mycobacterium leprae, Rickettsia prowazeki, Myobacterium tuberculosis, Vibrios cholerae, Corynebacteria diptheriae, Clostridium tetani, Shigella dysentriae, Treponema pallidum. This era saw also the publication of the malaria life cycle and Gram's staining technique.

## *CONCLUSION*

From this short treatise it is seen that the microscope is a child of evolution. While in some aspects the microscope has reached near perfection new applications of its princples are still being evolved and with techniques such as phase-contrast microscopy and instruments of electronic design the full benefits to

be derived from the microscope have not yet been fully realised and future years will see many new and startling developments and discoveries.

*A SELECTED BIBLIOGRAPHY*

Munoz and Charipper, The Microscope and Its Use Chem. Pub. Co., N.Y.

Rooseboom, Microscopium, Leiden.

Stephanides, The Microscope, Faber and Faber.

Wredden, The Microscope. Its Theory and Applications, Churchill.

## LIBRARY NOTES

In this issue we begin the Library Service, instituted sometime ago by a series of book reviews designed to help our trainees to develop a wider view of the subjects they study in the laboratory. It is unfortunately very easy to be insular in our attitude towards bacteriology, biochemistry, etc., as we see them practised in our daily work and to forget that they may be seen in their true perspective only when viewed against the background of history and science in general.

The librarian will be most grateful to receive helpful suggestions from both trainees and those concerned with supervising training programmes.

"FLEMING", by L. J. Ludovici

It has been said by some that Alexander Fleming was just another scientist who found fame by a lucky accident and that the credit for the wonder drug penicillin should go to those who developed it from the crude mould. It cannot be denied, however, that the discovery of penicillin was but the climax to a lifetime of valuable research into infectious diseases.

In this excellent biography of Fleming, we see that the knighthood and world acclaim bestowed on this quiet little man were well earned.

"PENICILLIN", by Alexander Fleming; publishers Butterworth's.

Published in 1946 and edited by Fleming, this book is a symposium on penicillin in the early days of its use. Many world famous authorities have contributed articles concerning the history, chemistry and use of penicillin in a wide range of infections. Perhaps the most interesting chapters from the point of view of the bacteriologist are those written by Fleming himself—"History and Development of Penicillin" and "Bacteriological Control of Penicillin Therapy."

In the light of present-day experience some of this material is outdated, but the book provides a very interesting and well rounded account of the problems and the work involved before a new drug can be made available for general use.

"MICROBES BY THE MILLION", by Hugh Nicol; publishers Pelican Books.

In the hospital we associate the microbe with corruption and disease. Dr. Nicol, formerly of the Rothamsted Experimental Station shows us in this book a new and fascinating world in which the microbe is seen as an essential member of the recurring cycle which provides us with food and life.

He deals with such varied topics as the heated haystack, fairy rings, your compost heap and actinomycetes in the perfume industry.

"VIRUSES AND MAN", by F. M. Burnet; publishers Pelican Books.

The study of viruses is one of the newest and most interesting branches of medicine and who is better qualified to discuss this subject than F. M. Burnet. As Director of the Walter and Eliza Hall Institute of Medical Research and Professor of Experimental Medicine in the University of Melbourne, he has made a major contribution in many branches of virology, especially the study of influenza.

In "Viruses and Man" he gives an account of the common virus diseases of man and discusses such questions as—how has the mumps virus survived unchanged since the days of Hippocrates?—where do influenza viruses go in summer?—is another pandemic like that of 1918 still possible? —Why has the poliomyelitis virus become important only within the last fifty years?

"Fleming", "Microbes by the Million", and "Viruses and Man" are available through any public library.

"Penicillin" may be obtained by sending 3d. in stamps to the Librarian:

Mrs M. B. COREY,
C/o. Pathology Department,
Christchurch Hospital.

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## N.Z. ASSOCIATION OF BACTERIOLOGISTS (INC.) AUCKLAND BRANCH CONFERENCE

Report on the Conference held in the Medical Centre, Auckland Hospital, on Saturday, June 4. This Conference was organised by the Auckland Branch of the N.Z.A.B. (Inc.).

The Conference was attended by some 40 to 50 people and included visitors from Whangarei and Thames Hospitals.

The Conference was opened by Dr. S. Williams, Pathologist, Green Lane Hospital. Dr. Williams included in his opening talk a few remarks about the Pathologists Sub-Committee which has been set up to look into the problem of bacteriologists' examinations and training.

The morning session was devoted to anticoagulant control and therapy. Papers were given by Drs. Kellaway and Fischman, Mr Davis and Mr Kennedy.

Dr. Kellaway covered the diagnostic and short term therapy side of the subject while Dr. Fischman covered E.C.G's and long term therapy.

Mr Davis covered Transaminase levels and Mr Kennedy the one stage clotting time.

In the afternoon papers were given by Mr Meredith—"Sweat electolytes in fibrocystic disease".

Mr Fischman "Hydatid serology". These were followed by a film on Tuberculosis lent by Pfizer and Co.

The papers were all of a high standard and enthusiastically received.

In the evening a highly successful cocktail party was held. It is hoped to make this Conference an annual event in future.

(Signed) R. KENNEDY,
Chairman.

## MINUTES OF A COUNCIL MEETING OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS (INC.) AT WELLINGTON ON APRIL 30, 1960

*Present:* Messrs Reynolds, Olive, Bloore, Donnell, Hutchings, Cameron, Lynch, and Rose. Miss Evans was absent overseas.

*Minutes of the Previous Meeting:* Moved: That these be taken as read. Olive/Bloore.

*Business Arising from the Minutes:* None.

Moved: That the Minutes be accepted and approved Olive/Donnell.

*Application and Resignations:* To be brought forward on to Agenda of Annual General Meeting that Mr Olive as Council Member express good wishes and propose Mr Josland as an Honorary Member.

Moved: That the application for membership be confirmed and the resignation be accepted with regret.

*Treasurer's Report:* The Treasurer pointed out the large number of outstanding subscriptions.

Moved: That all members receive annually a notification that current subscriptions are due and that in this notification any arrears be stated. Olive/Cameron.

Moved: That the Treasurer be authorised to pay expenses of the meeting. Lynch/Olive.

*Journal Report:* The Co-Editor, Mr Rose, reported that Miss Evans was due to return in April, 1961, and had expressed a wish to take up again the co-editorial work. Mr Rose again pointed out lack of articles.

*Inward Correspondence:*

Moved: That the following should compose the examiners' panel for the Final Examination: Messrs D. Whillans, J. Murray, H. Bloore, G. McKinley, H. Olive, L. Reynolds, and that the Secretary of the Laboratories Advisory Committee be notified accordingly. Donnell/Hutchings.

The Secretary was requested to reply to the Australasian I.M.L.T. pointing out the anomaly of "Australasian" and answering his queries.

In view of negative reply from Director-General of Health, Secretary requested to write to Minister of Health.

Moved That Inward Correspondence be accepted and that outward correspondence be approved. Cameron/Donnell.

*General Business:*

Moved: That the Intermediate Examiners' panel be amended and that the following should make up the Examiners' Panel:—Mr Adamson, Mr Rush-Munro, Mr Ekdahl, Mr Ellison, Mr George, Mr Hutchings, Mr Harper, Mr Schwass, Mr Philips, Mr Hilder. Carried. Donnell/Olive.

The Secretary was instructed to write to the Honorary Secretary N.Z.A.B., Auckland Branch, suggesting that he write on behalf of all recently appointed trainees who had been Laboratory Assistants to the Director-General of Health enquiring what proportion of Laboratory experience is to be allowed as training time.

A lengthy discussion took place on examination, reciprocity and as to how the general standing of the examinations and the Association might be improved.

Moved: That a sub-committee consisting of Hutchings, Donnell and Olive represent the Association to meet the Society of Pathologists at their coming meetings. Donnell/Olive.

The meeting closed at 4.30 p.m. with thanks to the chair.

## MINUTES OF THE MEETING OF THE COUNCIL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS (INC.) HELD IN CHRISTCHURCH ON JUNE 29, 1960

*Present:* Messrs Reynolds, Rose, Donnell, Bloore, Philip, Cameron, Lynch and Hutchings.

*Apologies:* An apology was received from Mr Olive.

*Minutes:* Minutes of the last meeting. Moved that they be accepted and approved. Donnell/Bloore.

*Applications and Resignations:* The Secretary was instructed to write to the Laboratory Assistants employed by Cooper McDougall and Robertson that their applications were still under consideration.

Moved that the applications for membership be confirmed and that the resignation be received with regret. Donnell/Lynch.

Mr J. Walker was invited into the meeting. He reported that business of the Conference was well in hand.

*Treasurer's Report:* Moved that the Treasurer be authorised to pay expenses of the meeting in the usual manner.

Moved that expenses of the Secretary and the President to the Conference where the above were not Hospital delegates be paid by the Association. Bloore/Cameron.

*Journal Report:* Mr Rose reported that some difficulty in assessing the class to which the Essays belonged was met with because of insufficient headings on the essays. There were nine essays and one technical essay.

Moved that because of insufficient entries in the technical section of the essay competition there be two prizes for the competition as a whole. Donnell/Bloore.

The first prize went to Mr McHardy, of Wellington, for his essay entitled "The Development of the Modern Microscope."

The second prize went to Mr K. R. James for his essay entitled "A brief outline of the Nature, Development and Discovery of the Bacteriophage."

*Correspondence:* Mr Reynolds reported on an offer by Biological Laboratories to give a prize to the writer of the best article published each year.

Moved that the offer of an annual prize to be known as the Rex Aitken Memorial Prize be accepted with thanks. Reynolds/Donnell.

The Secretary was requested to write to Mr Dunlop, of Hastings, suggesting that he refer his problem again to the Laboratory Advisory Committee through his resident Pathologist.

Moved that the inward and outward correspondence be received and approved. Philip/Donnell.

*General Business:* Considerable discussion on the formation of the sub-committee to meet the similar sub-committee of the Society of Pathologists ensured. It was decided that as the committees at this stage were to merely collect and collate information the present sub-committee which was to have met the Pathologists at their recent conference should remain, pending the approval of the incoming council.

The members of this sub-committee were Messrs Olive, Hutchings and Donnell.

Further discussion of the remits for the following day followed at some length.

The meeting closed at 9.30 p.m.

## MINUTES OF THE SIXTEENTH ANNUAL GENERAL MEETING OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS (INC.) AT CHRISTCHURCH, JUNE 30, 1960

Dr. L. C. L. Averill, Chairman to the North Canterbury Hospital Board, extended a warm welcome to the delegates and wished them well for the Conference.

Dr. D. T. Stewart, in opening the Conference, gave a stimulating and encouraging address. He titled his address "Women and Money." He described the difficulties encountered as a result of having to employ such a large number of women in laboratories and how after or during training so many were lost irrevocably to marriage or overseas travel. The man, on the other hand, was required to make his work his life-long interest—but was there, he asked, adequate encouragement for him to do so—was there an adequate reward for the skill required. Dr. Stewart went on to describe the unsatisfactory position in the grading of qualified staff holding positions of responsibility and as a result the inadequate recognition of this responsibility in the salary scale. Using examples to hand, Dr. Stewart strongly underlined the points of his address.

The President thanked Dr. Stewart for an address which reflected closely the feelings of the Association.

*Roll Call:* The following delegates were presented at the Conference:— Miss N. Davies (Waikato); Sister M. Paula (Auckland); Miss J. Montgomerie (Palmerston North); Mr N. I. Campbell (Waikato); Mr M. T. Lynch (Wellington); Mr D. C. Smith (Tauranga); Mr I. R. Buxton (Oamaru); Miss D. E. Hitchcock (Wellington); Miss J. R. Perry (Wellington); Mr A. Russell (Westport); Mr R. Wales (Napier); Mr F. C. Garnham (Napier); Mr J. A. Barrall (Hastings); Mr B. McLean (Christchurch); Mr A. H. Harper (Wanganui); Mr I. W. Saunders (New Plymouth); Mr G. D. C. Mears (New Plymouth); Mrs M. B. Corey (Christchurch); Mr F. L. N. Corey (Christchurch); Miss F. Wright (Timaru); Mr I. C. King (Auckland); Mr R. Kennedy (Auckland); Mr K. A. G. Watts (Auckland); Mr I. W. Cole (Auckland); Mr J. Sloan (Auckland); Mr A. Fischman (Auckland); Mr M. G. Jenner (Waipukurau); Mr R. Barrington (Hawera); Mr R. W. Smail (Invercargill); Mr G. C. Thompson (Invercargill); Miss R. Allen (Wellington); Miss M. Kennedy (Wellington); Miss C. B. Curtis (Christchurch); Mr G. Tait (Wellington); Mr W. Joyce (Lower Hutt); Mr K. G. Clarkson (Lower Hutt); Miss M. Lindsey (Auckland); Mr R. Curtis (Auckland); Mr J. P. Walsh (Auckland); Mr D. G. Till (Wellington); Mr V. J. Hawke (Nelson); Mrs J. A. Hough (Christchurch); Miss J. M. Christensen (Christchurch); Mr H. T. G. Olive (Wellington); Mr J. A. Walker (Christchurch); Mr B. W. Main (Christchurch); Mr D. J. Philip (Auckland); Mr M. McL. Donnell (Auckland); Mr H. G. Bloore (Blenheim); Miss M. Olsen (Palmerston North); Mr B. Robertson (Thames); Mr B. O'Meara (Rotorua); Mr J. H. A. Ward (Timaru); Mr G. R. Rose (Christchurch); Mr D. Adamson (Christchurch; Miss H. J. Rawson (Christchurch); Miss M. H. F. Cox (Christchurch); Miss J. Kelman (Christchurch); Mr R. D. Allan (Dunedin); Mr J. D. R. Morgan (Dunedin); Mr H. C. W. Shott (Dunedin); Mr W. Aldridge (Balclutha); Mr M. Poole (Wallaceville); Mr F. C. Kershaw (Dunedin); Mr T. J. Lewis (Whangarei); Mr N. D. Johnston (Kaitaia); Mr K. G. Reeve (Dannevirke); Miss V. M. Tucker (Christchurch); Miss

J. M. MacGibbon (Christchurch); Miss P. Scarf (Christchurch); Miss M. M. Eales (Christchurch); Miss S. Sinclair (Christchurch); Mr W. B. H. Gibson (Christchurch); Mr G. W. McKinley (Waipukurau); Mr M. R. Morris (Clyde); Miss L. Scarth (Christchurch); Miss D. M. Moylan (Ashburton); Mr G. A. Kuru (Wairoa); Mr L. Reynolds (Wellington); G. Cameron (Auckland); Miss J. Mattingley (Wellington); Mr J. Horner (Blenheim).

*Apologies:* Apologies were received from Mr N. Ellison (Wellington); Mr J. Murray (Christchurch) and Mr Ronald (Whangarei).

*President's Address:* Mr Reynolds expressed the regret of the Association at the death of Mr R. Aitken, of Auckland. Mr Aitken had been well known and well liked and his passing was a great loss to the profession.

Mr Reynolds expressed his best wishes to Miss L. Evans who is travelling overseas.

The invitation of the Ninth Science Congress, said Mr Reynolds, to participate was turned down with regret as no demonstration was available.

The President commented briefly on the visit of Mr Norman, one of the founders of the I.M.L.T. An illness had unfortunately stopped Mr Norman attending a Conference but he had been able to attend an examination as an observer.

Mr Reynolds expressed the need for all delegates to the Conference to convey to their staffs all information possible about the Conference, particularly concerning the desire of the Association to improve the examinations and status of the Hospital Bacteriologists. The need to encourage the junior members in the interests of the Association was evident.

All delegates were asked to express on paper their opinions and ideas as to how the status of Hospital Bacteriologists might be improved and send these to one of the three members of the sub-committee meeting a similar sub-committee from the Society of Pathologists. Mr Reynolds outlined briefly an example; a scheme which might be followed in the training of trainees.

*Proxies:* Proxies were received for Mr Kennedy from Auckland (35); Mr Walker, Christchurch (7); Mr Barrington, Hawera (1); Mr Hutchings, Palmerston North (1).

*Minutes of the Previous Meeting:* That these be taken as published. Olive/Donnell.

*Annual Report:* The Honorary Secretary presented the Annual Report. Moved that the Annual Report be adopted. Hutchings/Donnell.

*Journal Report:* Mr Rose requested that members publishing articles consider The Journal of the N.Z.A.B. first.

*Essay Competition:* First prize went to Mr McHardy, of Wellington. Second prize went to Mr K. R. James, of Hamilton.

*Balance Sheet:* The Treasurer presented the Balance Sheet. Moved that the Balance Sheet be adopted. Philip/Walsh.

*Election of Officers:* The following were elected to office:—

*President:* Mr H. Olive, Wellington.

*Vice-Presidents:* Mr H. Bloore, Blenheim; Mr M.McL. Donnell, Auckland.

*Secretary:* Mr H. Hutchings, Palmerston North.

*Treasurer:* Mr D. Philip, Auckland.

*Council Members*: Mr G. Cameron, Auckland; Miss J. Mattingley, Wellington; Mr J. Walker, Christchurch; Miss P. Scarf, Christchurch (Junior Member).

Mr Reynolds expressed appreciation on behalf of the members to the retiring Council members, Miss L. Evans and Mr M. Lynch.

*The Retirement of Mr. Josland:* Mr Olive spoke of the high standing held by Mr Josland and of the high regard felt for him by the Association.

Moved that Mr Josland be asked to rescind his resignation and accept appointment as an Honorary Life Member of the Association. Olive/Till.

*General Business*: Moved that the honoraria remain the same.

McKinley/Bloore.

Moved that the offer made by Mr Callaghan on behalf of Biological Laboratories of a prize to be known as the Rex Aitken Memorial Prize, for the best work published in each year be gratefully accepted.

Reynolds/Bloore.

Remit re Amendment to the Rules—

Mr Main spoke on behalf of the remit saying that it was felt that the Association had reached a size when the alterations of the members needed for the call of a Special General Meeting and for the formation of a quorum was more in keeping with the Association's membership.

Moved that Rule 13 para (d) be amended by the insertion of the word ten (10) instead of (5) and that para (f) of the same rule be amended by the insertion of the word thirty (30) in place of (10).

Main/Adamson. Carried.

Remits from the Auckland Branch of the Association were as follows:—

1. The Council of the N.Z.A.B. investigate the possibility of obtaining triple time rates for public holidays.

2. The Council of the N.Z.A.B. investigate the possibility of obtaining penalty rates for Graded officers.

3. That the Council of the N.Z.A.B. write to the Minister of Health setting out that because of the complete lack of realistic policy in its determinations, which are seriously affecting the senior staffing of Hospital laboratories that this Association declares a vote of no confidence with the Grading Committee.

4. That the Council of the N.Z.A.B. agitate through all possible channels open to it, to have the constitution of the Grading Committee amended so that there will be one bacteriologist from a Hospital Board and one bacteriologist not employed by a Hospital Board on the Grading Committe, and that the Council also investigate the possibility of the setting up of an Appeal Committee separate as a body from the Grading Committee where bacteriologists may lodge their appeals against the Grading Committees' determination where they feel there has been an unjust determination on their case.

Speaking on behalf of No. 1, Mr Kennedy expressed the difficulties in obtaining adequate staff for public holidays.

Mr Gibson felt that aims to make general financial improvements must come first. He was supported by Mr Adamson.

The remit was put to the meeting but failed to gain a two-thirds majority in favour.

Remit No. 2. Mr Bloore pointed out that a letter from Mr Mason, Minister of Health, in reply to submissions made by the Association in 1959, had in fact refused penal rates.

The remit was put to the meeting but was not carried by the two-thirds majority required.

Remits 3 and 4. Mr Kennedy asked that remits 3 and 4 be combined. He was seconded by Mr Watts. Mr Kennedy spoke on the remits basing his arguments on the situation as experienced in Auckland.

After some discussion the remits were withdrawn and the following motion substituted:—

That the Secretary be instructed to write to the Director-General of Health from the Annual General Meeting of the New Zealand Association of Bacteriologists expressing dissatisfaction with the Hospital Bacteriologists Salaries Grading Committee, its decisions and its present constitution.
Bloore/McKinley.

Change of Name:

The Secretary put the following motion:—

That the name of the New Zealand Association of Bacteriologists (Inc.) be changed to The New Zealand Institute of Medical Laboratory Technology (Inc.). Hutchings/Donnell.

Mr Bloore felt that in view of a statement in The Medical Supplements Bill in England where the Minister when asked had said that members of the I.M.L.T. would be called Technicians and not Technologists it would follows that we too would become "Technicians" and that the difficulties associated with the name would follow.

The motion was put to the meeting and was carried.

Conference, 1961:

Invitations were extended by Mr Saunders for New Plymouth and from Mr Kennedy for Auckland.

By vote, the invitation from Mr Saunders, of New Plymouth, was accepted.

Moved that the voting papers be destroyed. Hutchings/Walsh.

Mr Barrington enquired whether some form of newsletter containing information as to developments within the Association could be distributed during the year. It was generally felt that distribution of Conference news was dependent on the senior members and delegates to the Conference. It was decided to bring the matter before the incoming Council and the Journal Committee for their discussion.

Moved that the Secretary be instructed to write to the Minister of Health to ask whether members of the New Zealand Association of Bacteriologists are to be included in the proposed salary increases published in the press. Bloore/Olive.

Mr Reynolds expressed thanks and the appreciation of the meeting for the generous donations made to the Conference by Davis Gelatine, N. M. Peryer, William R. Warner, and Salmond and Spraggon and to Agfa for the loan of a fine projector, and also to those participants in the excellent trades display.

Mr Reynolds expressed thanks to the Conference Committee for their efforts towards the running of the Conference.

Mr McKinley spoke on the retirement of Mr Reynolds as President and expressed appreciation of the Association on his fine efforts on behalf of the Association. Moved a vote of thanks to the chair.

The meeting closed at 5.20 p.m.

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## RETIREMENT OF MR S. W. JOSLAND

On the 21st of March, Mr S. W. Josland, Chief Bacteriologist at the National Health Institute, was farewelled by a gathering of the staff of the institute and other friends from laboratories in Wellington.

Mr Josland's invaluable help and advice will be missed by many people in laboratories throughout New Zealand.

We would all join in the good wishes expressed at the Annual Conference and wish Mr Josland a happy retirement.

## ODE TO MR J

The wilting salmonellae wave
Flagellae in a long farewell,
And grieving leptospira grave,
With all forms of bacterial cell,
Gaze sadly round the vacant bench.
And realize with many a wrench
That nevermore will Mr J.
With their reactions still make hay.

Down in the houses the squeaks
And clucks of guineapig and hen
With noises from the rabbits, seek
To drown those of the sheep and men.
This matrimonial agency,
Where, at odd hours, so often he
Has been called down for many a task.
In vain for Mr J they ask.

No more within those walls confined,
And tended to through many a day,
Will trusting does with all their kind,
Brought up on pellets, greens and hay,
Look to the understanding touch
Of Mr J. within their hutch.
These cares, with others he has left,
And they like others are bereft.

The boiler house, funereal black,
The last time locked upon his round;
The volumes which a binding lack;
The order book, where is it found?;
The classroom where his saws were spoken;
The storeroom—please list lost or broken.
Now other hands must carry on
Alas when Mr J. is gone.

Arm, arm, ye brave, ye Taupo trout!
Look to your camouflage and speed.
Resist the lure before your snout,
The feathered thing above the weed.
Beware the shadows past the noon
Or morning's sunlight late or soon.
The freedom now of Mr J.
May spell your doom for many a day.

Long may the mountain snows look down
On Taupo's lake and Taupo's streams.
Long may the rod be bending there,
Fulfilling many of his dreams.
For us it's not a cheerful day
To say good-bye to Mr J.

—Dr. H.T.K.

## INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Examiners: Dr. J. F. Gwynne, Mr H. E. Hutchings.

Tuesday, 12th April, 1960, 09.30 a.m.-12.30 p.m.

### WRITTEN EXAMINATION

Time allowed: Three hours.

(All questions carry equal marks.)

1. You are working in a small district laboratory, and are required to send the following specimens to a base hospital:—
   (a) a section of large bowel for histology;
   (b) a sample of urine for 17-keto-steroids;
   (c) serum for Wasserman and Kahn;
   (d) serum for calcium estimation.
   How would you collect (excluding (a) and prepare them?
   How would you wrap and label them?
   What postal regulations govern their despatch?
2. Briefly give the meaning of the following terms.
   Answer only six.
   (a) Anti-human globulin.
   (b) Thrombocytopenia
   (c) Incomplete Antibody
   (d) Hypertonic solution
   (e) Rouleaux formation
   (f) Titre
   (g) Inactivation of serum
   (h) Antigen
   (i) Leucopenia
   (j) Pyrogen
3. You are given a swab and tissue from the interior of a cavity in a bone. Describe the procedure you would follow in order to detect the presence of an organism. Include details of culture media you would wish to use.
4. In performing a platelet count—
   What stains and solutions would you use,
   How would you collect your specimen?
   How would you count the platelets?
   Discuss the sources of error in your method. How may they be minimised?
5. Describe briefly how you would—
   (a) estimate protein in a C.S.F. sample.
   (b) test a urine sample for bile pigments.
   (c) estimate the quantity of albumen in urine sample.
   (d) detect occult blood in a faeces specimen.
6. Write brief notes on—
   (a) mycelium; (b) urinary casts; (c) Diagnex test; (d) E.D.T.A.; (e) bacterial spores; (f) Bence-Jones protein.

Tuesday, 12th April, 1960, 2.30 p.m.-5.30 p.m.

### *PRACTICAL PAPER A*

### BACTERIOLOGY AND BIOCHEMISTRY

1. Swab (A) is from an infected wound. Identify the organisms as far possible, using direct and cultural methods. Include sensitivity tests on the primary culture, using the antibiotic discs provided. Complete this question in Paper B (tomorrow). (Staphylococcus aureus, Coagulase Positive. Insensitive to Penicillin.)
2. Swab (B) is from an inflamed throat. Identify the organisms. If necessary, complete this examination in Paper B (tomorrow). Pneumococcus.)

3. Write notes on the four articles of equipment displayed.
   1. Volumetric Flask.
   2. Edwards Water Pump.
   3. Wide Range Capsule.
   4. Slide Micrometer.
4. Estimate the chlorides in the C.S.F. (C) provided.
5. Estimate the sugar concentration of the blood sample (D) provided. Give brief details of the steps in your procedure.

Wednesday, 13th April, 1960, 09.30 a.m.-12.30 p.m.

*PRACTICAL PAPER B*

BACTERIOLOGY AND HAEMATOLOGY

1. (a) Complete Question 1 from Paper A.
   (b) Complete Question 2 from Paper A.
2. Perform a reticulocyte count on the oxalate sample (E). Briefly what does your result imply?
3. (a) Stain the smear (F) from a sputum by the Z N method and report on the organisms. (M. tuberculosis.)
   (b) Stain the smear (G) by the Gram method and report on the organisms. The smear is from a purulent C.S.F. (Meningococci.)
4. (H) is a sample of blood from a woman attending ante-natal clinic during her third pregnancy.
   Group and Rh type the sample . (Blood group B, Rh (D) Negative.) State what further investigations you consider should be done.
5. (a) Make a Pasteur pipette from the piece of Quill tubing supplied.
   (b) Plug the test-tubes supplied with cotton wool.
   (c) Pour a plate from the molten agar supplied.

The following candidates were successful:—

Miss C. M. C. COX, Hamilton.
Miss R E. SAMUELS, Napier.
Miss T. G. LOGAN, Hamilton..
Miss R. H. BRIANT, Gisborne.
Miss J. M. WITTAM, Rotorua.
Miss M. J. STEWART, Rotoura.
Miss D. M. MOYLAN, Ashburton.
Mr P. A. JONES, Tauranga.
Miss B. J. FURNESS, Nelson.
Miss S. A. NIELSON, Wanganui.
Mr T. J. LEWIS, Whangarei.
Miss L. McINTOSH, Dunedin.
Miss E. BLACKIE, Buller.
Mr R. C. SOWDEN, Auckland.
Miss A. C. ELLETT, Auckland.
Mr D. F. MITCHELL, Auckland.
Mr K. M. K. BECKETT, Auckland.

## INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Wednesday, 27th, April 1960

Examiners: Dr. M. McKellar, Mr H. Bloore.

*WRITTEN EXAMINATION*

Time allowed: Three hours.

Answer all questions: *All questions carry equal marks.*

QUESTION 1:

Give a detailed account of a method for estimating C.S.F. Chlorides, including the method of standardising the reagents where necessary.

QUESTION 2:

Describe in detail four methods of growing organisms under anaerobic conditions.

QUESTION 3:

Many anticoagulants are used in laboratories for blood and other samples.

Name as many as you can and give the following information about each one.

(a) Purpose for which used, i.e., which test.
(b) Any purpose for which it should NOT be used and why.
(c) How each anticoagulant prevents clotting.

NOTE: After stating name of the anticoagulant, give the required details under separate headings:—(a), (b), (c).

QUESTION 4:

Describe fully the stages in the microscopic examination of a peripheral blood film. Detail the microscopic findings you would expect to observe in the peripheral blood film of a patient with severe pernicious anaemia.

QUESTION 5.

Write brief notes on the following:—

| | |
|---|---|
| Uncorrected leucocyte count | Stippled cells |
| Autoantibody | Myeloblast |
| Isoantibody | Target cell |
| Buffy coat | Hypersegmented polymorph |
| Clot retraction | Heterophil agglutinin |

QUESTION 6: 20 questions *only* to be answered.

*Note*: Answers here are to be very brief.

1. What is the name of the pigment which gives normal urine its colour?
2. Name two other pigments found in urine in disease.
3. Name three methods of detecting albumin in urine.
4. Name the ingredients of Benedict's qualitative reagent.
5. What reducing sugars may be found in urine—name two?
6. Give the range of urine S.G. found in health.
7. Name four organisms which may cause, and commonly do cause, cystitis.
8. Note typical laboratory findings in the C.S.F. in T.B. meningitis.
9. Note typical laboratory findings in the C.S.F. in meningococcal meningitis.
10. What is Pandy's reagent and what is it used for?
11. What is the principle of the method you employ for estimating C.S.F. protein?
12. Outline your method of performing a cellcount on a C.S.F., including the calculation.
13. What is xanthochromia and how is it produced?
14. What test is widely used to gauge the pathogenicity of staphylococci, and oultine your method of performing it?
15. Name four common characteristics shared by organisms of the Salmonella and Shigella groups.
16. Culturally, what are the two main differences between Salmonella and Shigella?
17. State some characteristics (morphological, cultural and biochemical) of B. coli, B. pyocyaneus and B. proteus, under the headings Similarities and Differences.
18. Outline the principle of the mode of action of a McIntosh and Fildes Jar.

19. Name the ingredients of the medium you use to grow M. tuberculosis.
20. Name the ingredients of the medium you normally use to plant pus swabs on.
21. What method do you employ to grow H. influenzae?
22. What are the main characteristics of the organisms causing gas gangrene and name two members of the group?
23. State the differences between O and H agglutination, under the headings:—
    (a) Appearance.
    (b) Duration of incubation usually employed.

Wednesday, 27th April, 1960, 2.30 p.m.-5.30 p.m.

BACTERIOLOGY

QUESTION 1:

Identify as far as possible, the organism provided. This organism was isolated from a specimen of faeces. List the tests you perform, together with the results in each case. (Proteus rettgeri (non-swarming).)

QUESTION 2:

Examine fully the urine specimen provided. A urinary infection is suspected.

Tabulate your results, including drug sensitivities. (B. pyocyaneus.)

QUESTION 3:

Perform a Widal test on the serum provided. This is from a patient suspected of having ?? typhoid fever. The titre is required, and a brief outline of your technique (H antibody titre only).

BIOCHEMISTRY

QUESTION 1:

Estimate the blood sugar in the specimen provided, using Folin Wu's method.

QUESTION 2:

Find the normality of the potassium permanganate solution provided. Standard N/100 sodium oxalate solution is provided.

Thursday, 28th April, 1960, 09.30 a.m.-12.30 p.m.

*HAEMATOLOGY*

QUESTION 1:

Determine the compatibility of the donor blood 'Y' with the serum of the patient 'Z' provided. The patient is known to be pregnant.

Detail your technique briefly.

QUESTION 2:

Determine the ABO type of the blood sample 'X' provided.

Detail the technique briefly.

QUESTION 3:

Perform a leucocyte count and differential count on the blood 'A' provided.

Leave the film for inspection.

(Neutrophil Leucocytosis.)

QUESTION 4:

Report on the peripheral blood film 'B' provided.

(Cooley's Anaemia.)

ORAL EXAMINATION:

The Examiners asked the candidates to discuss the following in the Oral examinatons:—

*Dr. McKellar*:

Formed element of blood; Direct and Indirect Platelet Count; Buffy Coat Films; L. E. Cells; Definition of Macrocytosis; Significance of Rou-

leaux in Blood Films; Trapped plasma; Blood and haemoglobin in urine; Occult blood tests on faeces; Enzyme treated red cells; Fisher-Race nomenclature; Autoantibodies and isoantibodies; $D^u$; Basis of Coombs' Test; Albumin cross-matching; Gastric Analysis; Histamine; Total and Free Acid; Significance of target cells and Howell-Jolly bodies.

*Mr Bloore*:

Normal and Molar Soluction; pH; Volumetric pipettes; Cleaning of Glassware; Beer's Law; Methods of sterilisation; Principle of Autoclave; Bile pigments in urine.

The following candiates were successful:—

Mr G. D. HAINS, Hamilton.
Miss S. J. OLNEY, Napier..
Miss A. W. FOY, Tauranga.
Miss J. M. MAKIN, Auckland.
Mrs B. F. WALLACE, Auckland.
Miss P. C. A. SOMERS, Auckland.
Miss S. M., McMULLIEN, Whangarei.
Miss R. M. MACKIE, Wanganui.
Miss R. J. ASHTON, Timaru.
Mr T. J. NAUGHTON, Hastings.
Miss H. A. N. DACRE, Wairoa.
Mr K. H. BODDY, Dunedin.
Mr G. E. EVES, Auckland.
Miss J. M. McCLURE, Auckland.
Mr D. N. THORBURN.
Mr C. S. CURTIS, Auckland.
Miss M. M. BLACK, Wellington.

The Council of the N.Z. Association of Bacteriologists invite members to design a common seal for the N.Z. Institute of Medical Laboratory Technology. A prize of two guineas is offered for the accepted design. Closing date 1st October, 1960.

## WAIKATO HOSPITAL BOARD
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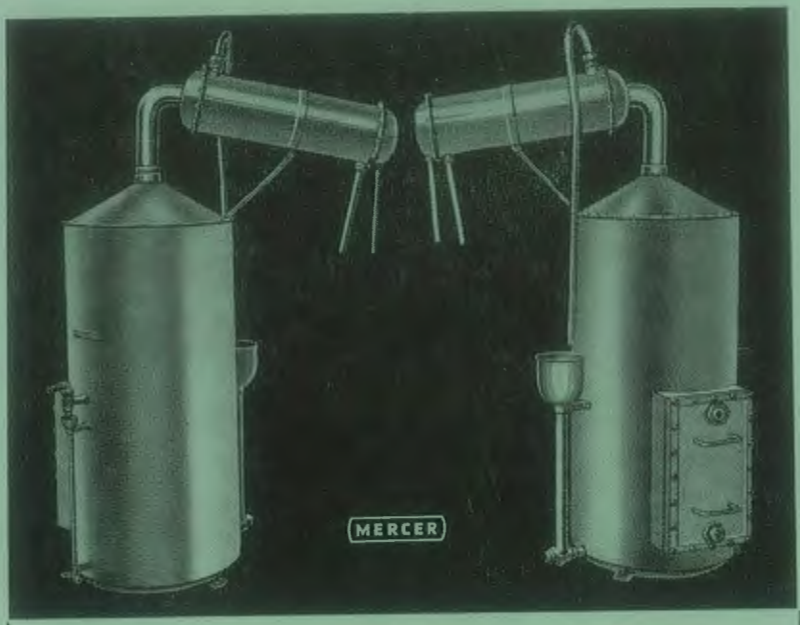


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