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JOURNAL

OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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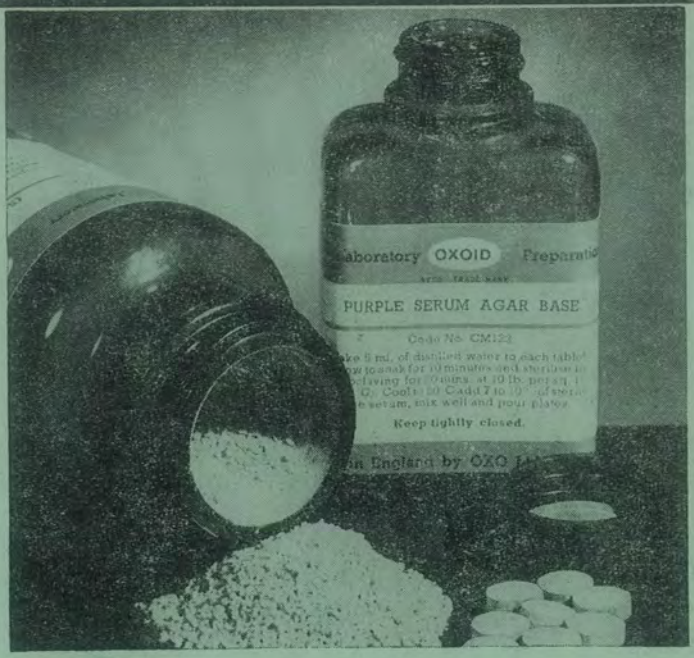

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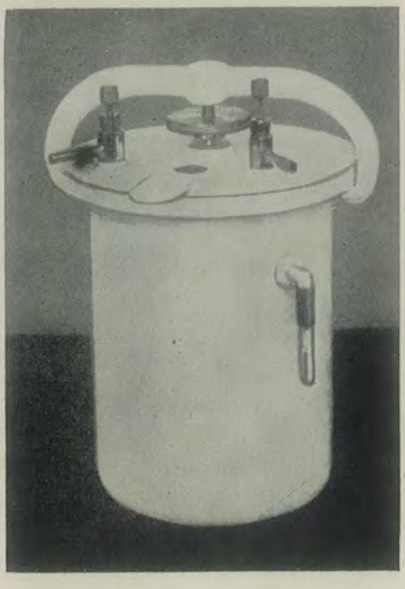

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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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Communications primarily affecting the Association should be addressed to the Secretary, Mr H. E. Hutchings, Pathology Department, Palmerston North Hospital.

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ADDRESSES

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THE DETERMINATION OF SIZE AND HAEMOGLOBIN CONTENT OF RED CELLS

ROBIN H. BRIANT

(*Cook Hospital Laboratory, Gisborne*)

(*Winner Essay Section, Junior Essay Competition*)

Measurement of red cell size and haemoglobinisation plays an important role in haematological diagnosis. Many anaemias are characterised by peculiar erythrocyte morphology and this can be demonstrated in a blood film examination or by actual measurement of cell properties. In this paper I intend to discuss some of the method used in making these measurements, and conditions affecting abnormal and inaccurate results.

INDIRECT MEASUREMENT OF RED CELL SIZE AND HAEMOGLOBINISATION

Methods easily applied to routine laboratory work are naturally the most popular. In 1929 Wintrobe advanced the system of absolute values which has now largely replaced the older system of indices. The difference between these two methods is fully expressed in their titles: 'absolute' meaning 'independent of a standard figure' and 'index' meaning 'as compared with a fixed standard'. For a full description of the calculations I refer the reader to Dacie's 'Haematological Technique'. There is opportunity here only to describe briefly the underlying principles of the absolute system.

Haemoglobin content of red cells must necessarily be measured indirectly, the weight per cell being expressed in units as small as micromicro-grams. The results of three routine haematological tests, the haemoglobin concentration in grams per 100 ml. blood, the volume per cent of packed erythrocytes and the count per cmm. of red-cell population, are necessary for measurement of mean cell haemoglobin (M.C.H.) and mean cell haemoglobin concentration (M.C.H.C.). Careful distinction between these two values should always be made—mean cell haemoglobin is a measurement of the actual haemoglobin content of the average cell whilst mean cell haemoglobin concentration is the ratio of the weight of the haemoglobin per cell to the cell volume. For reasons later to be mentioned the latter is the more valuable estimation.

Mean cell volume is calculated in a similar manner using results of the red cell count and packed cell volume.

It is important to note that anisocytosis is not revealed by this system. It is conceivable that a patient with marked variation in cell size, ranging from micro to macrocytosis may produce a substantially normal mean cell volume, the two extreme conditions

neutralising each other. For this reason the absolute values should never be used without a close inspection of the blood film. Despite its limitations this method is distinctly superior to that involving comparison with an assumed normal.

DIRECT MEASUREMENT OF RED CELL SIZE

Several attempts have been made to devise satisfactory methods of direct measurement. Almost without exception they are time-consuming and laborious. I shall mention here only three.

1. *Diffraction methods:*

'When white light is viewed through an evenly spread blood film, the light rays passing through the slide are diffracted by the opaque cells. Halo-spectra are so formed, the diameters of these being inversely proportional to the diameter of the cells.' This is the principle of a method widely used and with many applications. However, it is practicable only when there are sufficient numbers of cells of one particular diameter—with marked anisocytosis the halos are indistinct and difficult to match. Results of this method indicate the modal mean diameter (i.e., the size at the peak of the Price-Jones curve: below) rather than the arithmetic mean which is expressed by the absolute values. However, in a normal subject these figures should be almost identical.

2. *The Price-Jones curve:*

A great deal of haematological literature has been devoted to descriptions of the Price-Jones technique which is now seldom employed. Its results are interesting but provide little information that cannot be gleaned from an examination of the blood film. The process is, briefly, to project the magnified image of a stained blood film and measure the diameter of at least five hundred cells. The number of cells of each size is plotted on a graph and the degree of anisocytosis assessed by comparing the resultant curve with a normal one.

3. *Measurement in plasma:*

Ponder (1924) suggested that the drying of a blood film caused shrinkage of the erythrocytes and he advanced a method for measuring the cells suspended in their own plasma. Houchin, Munn and Parnell (1958) describe their application of this technique. The suspended cells form blocks of rouleaux and are thus photographed. A stage micrometer super-imposed on the film is used to measure both diameter and thickness. It appears that the packing together of the cells tends to decrease the thickness and increase diameter, but the final result of cell volume is un-

altered. The volume, according to Wintrobe the most valuable of the size measurements, is calculated from a formula which treats the cell as a spheroid.

NORMAL VALUES

I have found that no two texts agree on the normal range of these values. Different methods produce widely varying results, so it is rather difficult to define the limits—either normal or abnormal. Drawing from several sources I shall list the widest possible normal range (adult values), assuring the reader that values outside these should be regarded at least with suspicion.

Mean cell haemoglobin	24-35 micromicrograms.
Mean cell haemoglobin concentration	28-38%.
Mean cell diameter	6.6-7.6 microns.
Mean cell average thickness	1.7-2.5 microns.
Mean cell volume	74-106 cubic microns.
Diameter / thickness ratio	2.4: 1 - 4.2: 1.

ABNORMAL VALUES IN DISEASE

Organic disease in many forms is manifested in abnormal blood conditions and dysfunction of the bone marrow. An examination of the bone marrow is necessary in making a positive haematological diagnosis, but one can often predict the state of the marrow by the morphology of the erythrocytes in the peripheral blood. Macrocytes, for example, are usually liberated from an actively producing marrow which has an arrested maturation. Microcytes, on the other hand, are often produced by a normal or over-active marrow, striving to replace the total number of circulating erythrocytes following severe blood loss or in defective diet.

Such a condition as the latter would show considerable decrease in diameter, volume and haemoglobin content of the cells, whilst the mean concentration of haemoglobin per cell may be normal or only slightly lowered.

In Pernicious anaemia, unless the macrocytosis is extreme the mean cell volume and diameter may not be greatly increased. A study of the Price-Jones curve, however, should reveal a completely deranged pattern; the base being widespread, ranging perhaps from 4μ to 10μ, and the peak well above normal at about 8.6μ. Thus the modal mean diameter is raised somewhat more than the arithmetic mean. An interesting observation is recorded by Larsen (1952). He describes two distinct cell types present in the peripheral blood in Pernicious anaemia, one physiologically normal, the other with a tendency to increase in diameter and

decrease in thickness when spread in a blood film. This phenomenon explains the fact that the mean cell volume in this disease is not raised correspondingly with the mean cell diameter. Whitby and Britton (1953) describe the diameter/thickness ratio which is said to remain constant in Pernicious anaemia, and in fact, in almost all conditions except haemolytic anaemia with congenital spherocytosis.

In this last mentioned disease the red cells in a stained blood film do not show the central pallor of normal red cells. Whitby and Britton state that spherocytes are derived from erythroblasts of normal dimensions. The mature forms, however, have increased thickness and decreased diameter, normal volume and decreased surface area. Unless iron deficiency is present as well the haemoglobin content of the cells will be normal or raised. Spherocytes are the only type of red cell alleged to have an increased mean haemoglobin concentration.

In contrast to the above Whitby and Britton suggest that anaemia of obstructive jaundice has a decrease in cell thickness and increase in diameter. However, I have been unable to find any other evidence that such is the case.

ABNORMAL VALUES PRODUCED BY INACCURACY AND VARIATION

Abnormal values, in complete discord with the patients' state of health, may be encountered. One should remember that there are numerous technical and physiological factors which could influence variation from the normal. The most outstanding of these are listed below.

The accuracy of the absolute values depends almost entirely on the accuracy of the three subscribing estimations. The possibility of error in these tests has been well stressed in the past and there is no need for me to repeat any warnings to technicians undertaking these determinations. It is a common practice in many laboratories to calculate the absolute values on any patient whose haemoglobin percentage concentration is less than 11.0 grams. Although this is a wise precautionary measure to exclude the possibility of a complicated anaemia, I feel that unless the routine laboratory tests are carefully standardised and carried out with every attention to detail, slight inaccuracies may serve only to confuse the picture. For example, a correct value for packed cell volume coupled with an undercounted red cell population will present an untrue picture of macrocytosis. Since the red cell count done by the usual haemocytometer method holds the most room for error, the mean cell haemoglobin concentration, which does not involve this count, is the most reliable of the three.

In direct measurement of cell diameter and thickness by micrometry the scale must be carefully calibrated as any discrepancy will be greatly magnified. The red cells shrink when dried in a blood film and the degree of shrinkage varies with conditions of drying and staining. The specific cell types of Pernicious anaemia described by Larsen are said to shrink even more than normal cells. Wintrobe (1956) estimates the shrinkage of normal cells to be as much as 0.7μ - 1.0μ, and therefore recommends the measurement of cells in wet preparation.

Personal variations, made apparent by any of the methods described here should always be borne in mind by anyone attempting a diagnosis from values for red cell dimensions. Whitby and Britton have also found a gradual increase in cell diameter during the day, with subsequent decrease during sleep. The difference may be as great as 0.6μ and they suggest that this variation is inversely proportional to the alkalinity of the blood. Wintrobe says that despite the numerous possibilities for variation in red cell size, the cells do, in fact, remain surprisingly constant.

SUMMARY

Several methods, both direct and indirect, have been devised for making measurements of red cell size and haemoglobin content.

Values for red cell dimensions and haemoglobinisation are useful in making a positive haematological diagnosis.

The values alone are not diagnostic but must be used in conjunction with clinical history, results of other laboratory investigations and a close inspection of the blood film.

Sources of error and variation are numerous and must be considered along with the possibility of disease.

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D-CYCLOSERINE, A NEW ANTITUBERCULAR DRUG

MICHAEL J. LYNCH
(*Pathology Department, Wellington Hospital*)

(*Winner Technical Section, Junior Essay Competition*)

Since Ehrlich's discovery of salvarsan as a therapeutic agent in syphilis, there have been many attempts to find a chemotherapeutic drug for the treatment of tuberculosis. Metal salts, particularly calcium and gold compounds, have attracted most attention in the past. In 1938 Rich and Follis reported the inhibitory effect of sulphanilamide in experimental tuberculosis in guinea pigs. Investigations with sulphone compounds although promising with experimental tuberculosis gave very disappointing results with human tuberculosis.

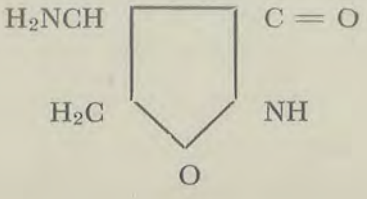
In 1941 Bernheim made observations of the inhibitory effect of benzoic and salicylic acid on the metabolism of tubercle bacilli. In 1944 Schatz and Waksman reported that they had obtained an antibiotic from the fungus Streptomyces griseus. A year later Youmans reported the antitubercular activity of streptomycin.

Of the drugs in use for the treatment of tuberculosis at the present time Streptomycin, P.A.S. and Isoniazid are the most common. But the most important problem in the use of these drugs in tuberculosis is the development of resistance of the tubercule bacilli towards them. With the increasing number of cases in which drug resistance was becoming apparent, new and more powerful antibiotics were sought.

In 1954 Harned discovered D-cycloserine, an antibiotic which was to prove valuable for the treatment of such cases. It is the aim of the author to present a brief history and summary of the properties of this new drug, D-cycloserine, with particular reference to the method of determining the *in vitro* sensitivity of M. tuberculosis.

CHEMISTRY

The antibiotic D-cycloserine was discovered by Harned in a culture of Streptomyces orchidaceus and by Harris in a culture of S. garyphalus in 1954. The active substance was found to be D-4-amino-3-isoxazalidinone which has a structural formula as follows:



Cycloserine is a white crystalline powder that is soluble in water at 25°C to the extent of 100 mg. per ml. Its melting point is 155°C and the molecular weight is 102. It is unstable in acid or neutral aqueous solution; it breaks down to form hydroxylamine and serine. In alkaline medium cycloserine is quite stable.

METHODS OF ASSAY

Cycloserine can be determined colorimetrically or microbiologically in body fluids or tissues. In a slightly acid medium cycloserine forms a blue complex with sodium nitroprusside with an intensity that can be read colorimetrically.

Method: To 2 ml. of protein free filtrate of the body fluid under test add 0.2 ml. of 12.6 N. acetic acid.

Mix. Then add 0.2 ml. of 5.6% sodium nitroprusside.

Mix well and after 15 minutes read in a Spectrophotometer at a wavelength of 625 millimicrons.

Standards are made up of normal serum in which 2 ml. contains 5 to 100 micrograms of cycloserine.

Among the microbiological methods of assay, nephelometric and diffusion methods (especially disc, cylinder and agar plate cup tests) are used. The usual test organisms are Staphylococcus aureus, Sarcina lutea, B. subtilis or M. tuberculosis.

STABILITY

In physiological saline containing 5% cycloserine colorimeteric determinations show 4% remaining after storage for 20 hours at 4-6°C. At room temperature only 1.6% remained. At pH 8.75 solutions are practically stable. At 0°C cycloserine remains fully active microbiologically for 10 days in sterile filtered human urine. At room temperature 0.1% aqueous solutions maintain tuberculostatic activity for three weeks, following which the activity decreases rapidly.

ANTIMICROBIC ACTIVITY

The results obtained by numerous investigators using a variety of methods show cycloserine to be an antibiotic with a wide spectrum of activity. Compared with other antibiotics, however, its activity *in vitro* is limited. Its chief manifestation is inhibition of growth; microbicidal action is normally obtained only with prohibitively high concentrations. It is noteworthy that of all the strains of bacteria so far tested Mycobacteria are the most sensitive. Cummings showed that the tubercle bacillus (strain $H_{37}Rv$) is sensitive to concentrations of 2.5 to 5 micrograms per ml. cycloserine. Partnode obtained complete inhibition of $H_{37}Rv$ with concentrations of 10 micrograms per ml. with or

without the addition of human serum. In the presence of guinea pig serum, D-cycloserine concentrations of 20 micrograms per ml. were required. It is therefore seen that the addition of serum reduces the effect of cycloserine, although human serum to a lesser extent. This anomally will be discussed later.

The growth of Mycobacteria is more strongly inhibited, especially initially in fluid media than on solid media. On incorporation in egg medium cultures before coagulation, quantities roughly 10 times as great are needed to inhibit the growth of tubercle bacilli as in fluid media. If cycloserine is dropped on to the prepared egg culture no such loss of activity is noted. In intermittent trials, contact with 5 to 200 micrograms of cycloserine per ml., Dubos' medium for 8 hours is sufficient to permanently prevent multiplication of tubercle bacilli. In tissue cultures, cycloserine, like I.N.A.H., inhibits not only extra cellular tubercle bacilli but also intra cellular bacilli. *In vitro* tubercle bacilli slowly disintegrate under cycloserine and loose to a greater or lesser extent their acid fastness.

Although D-cycloserine possesses an antimicrobic spectrum greater than the Mycobacteria group such properties are beyond the scope of this report.

EXPERIMENTAL TUBERCULOSIS

In experimental tuberculosis in animals Conzelman has shown that the activity of D-cycloserine varies widely and is in fact relatively ineffective. Because of the good response many other organisms have to cycloserine this failure with tuberculosis is totally unexpected. These differences appear to relate to blood concentrations and the time that the antitubercular concentration can be maintained. Experimentation has shown that blood concentration of 25 micrograms per ml. of cycloserine is needed for significant benefit. Such concentrations can be maintained in a monkey for 10 hours, whereas similar dosage in the guinea pig gives the same concentration for only 4 hours. It therefore appears that rapid urinary elimination may be a factor which would account for the lack of effect of the drug in laboratory animals.

CLINICAL NOTES

Although D-cycloserine has been shown to be relatively ineffective in experimental animals, even in high doses, it has been proved an effective compound for the treatment of human tuberculosis. Why this paradox? Several reasons have been suggested; for example, that there is a factor in the serum of man that is antagonistic to the tubercle bacillus and that cannot be found in

animals. A further suggestion is that the drug is altered in man to a substance having greater antitubercular activity; or finally that animals excrete the drug more rapidly.

In a preliminary trial using D-cycloserine Livings and Cummings noted the emergence of bacillary resistance to the drug and that in cases where the bacillus was already sensitive to Streptomycin and Isoniazid, treatment with cycloserine did not show a more rapid improvement of the condition. Other experimental work suggests that cycloserine in combination with Isoniazid shows greater therapeutic activity than either drug alone.

SENSITIVITY TESTS

Determination of the *in vitro* sensitivity of the tubercle bacillus to cycloserine is thus important in cases of bacillary resistance to the common drugs. It is the object of this paper to describe a method which has been employed to determine bacterial sensitivity. It is not suggested that this method is necessarily the best or the most accurate, but it was devised and adopted as the most suited to the resources of the general hospital laboratory.

LABORATORY PROCEDURE

Any standard method of digestion and concentration of the specimen is acceptable. The method used by the author is concentration by trisodium phosphate. An equal volume of 23% $Na_3PO_4.12H_2O$ is added to the specimen in a screw cap bottle. The bottles are well shaken and placed in the incubator (37°C) for 18-24 hours. The bottles are then removed from the incubator, a few drops of 0.1% bromo thymol blue is added, and 4N. HCl is added dropwise until the treated specimen is neutralised. The specimen is then centrifuged at 3000 r.p.m. for 20 minutes. The supernatant fluid is decanted and the remaining deposit is cultured. Any of the regular solid culture media may be used for the primary isolation.

When typical luxuriant, dry, crumbly, cauliflower-like colonies of M. tuberculosis are observed on the culture medium a large loopful representative of the growth is emulsified in about 0.5 ml. of sterile distilled water in a bijou bottle. After the larger particles have settled about 0.25 ml. of the suspension is transferred to a tube of Dubos medium (Difco.). This medium has a pH of 7.2 and contains bovine albumin (fraction V, Armour & Co.) of a concentration of 0.3%. These cultures are incubated at 37°C for 10-12 days to ensure a smooth diffuse growth. Adjustment of the culture to a standard opacity is unnecessary as increases or decreases in inoculum size were found to have little effect on the end point of the test.

The cycloserine was suitably diluted with distilled water such that when 0.1 ml. of the dilution is added to 4.9 ml. of Dubos medium final concentrations of 1, 5, 10, 50, and 100 micrograms per ml. are obtained. The dilutions of cycloserine to give these concentrations are prepared from a stock solution that is prepared freshly every three weeks. A bulk stock of these dilutions can be stored in the refrigerator at 0°C for three weeks before any loss of antitubercular activity is noted.

A series of tubes containing these concentrations are set up in a rack together with a tube of Dubos medium, as a control, and a tube of nutrient agar, as a control of the purity of the culture of tubercle bacilli. The tubes are inoculated with one drop (0.02 ml.) from a Pasteur pipette, of the 10-12 day culture and incubated at 37°C. The tubes are mixed and examined at frequent intervals for 10 days and the sensitivity of the strain is recorded as the lowest concentration of cycloserine to give complete inhibition of growth after 10 days' incubation.

A similar series of tubes are set up at the same time as the test series and are inoculated with a control strain of $H_{37}Rv$ and incubated under the same conditions. This will detect any variation in the activity of the drug due to storage or interference by the albumin fraction of the medium. It must be remembered that $H_{37}Rv$ is sensitive *in vitro* to 5 micrograms of cycloserine per ml. of medium.

All normal safety precautions connected with laboratory procedures using tubercle bacilli must be emphasised and only experienced technicians should carry out this work.

SUMMARY

An evaluation of the properties and the antimicrobial activity, especially towards M. tuberculosis of D-cycloserine has been presented. A method of determination of the *in vitro* sensitivity of M. tuberculosis to this drug has been described.

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A METHOD OF CONCENTRATING HYDATID HOOKLETS

D. H. ADAMSON

(*Pathology Department, Christchurch Hospital*)

The case under investigation was of Mr V.S., aged 59, in the Christchurch Hospital. He had had a hydatid cyst in the lung and one occupying almost the whole area of the right lobe of the liver removed in July, 1958.

In April, 1959, another hydatid cyst was removed from the edge of the liver. At the same time a small calcified cyst was removed from within the omentum in the left abdomen. The walls of this cyst could not at first be identified histologically as a hydatid owing to its fibrous nature and low cellularity. However, some time later, after decalcification, this was possible.

Wet films made from several areas on the inner wall and from the contents of the cyst did not reveal any hydatid elements.

An attempt was then made to concentrate any hooklets which may have been present in the cyst content which are rich in calcium phosphate and cholesterol (Dew, H.R., 1928). At first, 25% hydrochloric acid was used, in which one half of the cyst was immersed overnight and then vigorously shaken. Ten millilitres of this mixture was gravity-sedimented for one minute, then the supernatant was centrifuged at 2,000 R.P.M. for five minutes.

The deposit showed three hooklets of Echinococcus granulosus in one of two films. The recognition of these hooklets, which cannot be mistaken for any other structure, leaves no doubt as to the nature of a cyst.

The 25% acid was then changed for concentrated hydrochloric acid, in which the specimen was immersed for three days. The volume of the acid used was about five times that of the specimen.

Moderate numbers of hooklets, perfectly preserved, could be readily found in wet films of the centrifuged deposit from this concentrate.

Fresh concentrated hydrochloric acid was then added and the remains of the specimen were boiled for a few minutes in this. A little more digestion took place, but undamaged hooklets were still present in films from the deposit.

As hydatid hooklets are composed of chitinous material—a sort of carbohydrate, or "plastic", it is not really surprising that they survived this treatment. The procedure is only mentioned to draw attention to a satisfactory, proved method to aid in the identification of at least some hydatid cysts. There has not been opportunity yet to experiment with more than this one cyst, but a sputum has been very satisfactorily concentrated by a five minute treatment with twice its volume of concentrated hydrochloric acid. A refinement of the method may be adopted if one wishes to obtain a clearer preparation. This involves a final washing of the cyst-HCl. mixture with ether to remove cholesterol and fats. After centrifuging this mixture, the hooklets may be found in the bottom of the tube with very little other sediment.

As an experiment a large volume of "sand", i.e., scolices from a hydatid cyst in a human liver was treated with different concentrated acids overnight at room temperature.

The scolices and hooklets treated with concentrated sulphuric and nitric acids disappeared completely, as might be expected. The scolices treated with concentrated hydrochloric acid were completely dissolved with the exception of some which remained as faint ghost structures. The hooklets remained in perfect condition and sometimes in their original rosette formation within the ghosts.

This work was started at the suggestion of Dr. D. T. Stewart, Director of Pathology and Dr. G. C. T. Burns, Microbiologist.

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TRANSAMINASE AND DEHYDROGENASE ESTIMATIONS

MISS JOAN MATTINGLEY

(*Pathology Department, Public Hospital Wellington*)

The determinations of serum levels of transaminases and dehydrogenases have become important aids in the diagnosis of myocardial infarcts and liver damage. These estimations are based on the knowledge that some tissues are rich in such enzymes, and that necrosis of these tissues liberates significant amounts of enzymes into the blood where they can be measured. These estimations require great attention to the details which control enzyme activity, i.e., pH, time, temperature, concentration of reactants.

GENERAL PRECAUTIONS: Serum must be completely free from haemolysis. Red blood cells are rich in these enzymes, and the amounts found in normal sera are due to normal cell breakdown. Rough handling of blood or insufficient centrifuging leaving red cells in the serum result in artificially high results. Haemolysis can be particularly troublesome when specimens from patients on anti-coagulant therapy take hours or days to clot. It is almost impossible to get reasonable sera from such specimens.

Sera and reagents must be kept frozen until required. Serum left standing at room temperature can lose 10-20% of its enzyme activity in an hour. The quantities of reactants must be strictly adhered to. Rough weighing or pipetting can ruin these estimations. Buffer solutions must be accurate and temperature and reaction time carefully controlled, because the units in which results are expressed are defined according to these conditions.

Glassware must be clean, as enzyme reactions are easily poisoned by traces of contaminants.

LACTIC DEHYDROGENASE (referred to as L.D.H.): This enzyme catalyses the reduction of pyruvate to lactate;

$$\text{Pyruvate} + \text{DPNH} \rightleftharpoons \text{Lactate} + \text{DPN}.$$

The assistance of Coenzyme 1 (called DPN, or DPNH when in its reduced form) is necessary to act as the intermediary hydrogen acceptor. The reaction is a reversible one but usually lies to the right, because the reverse reaction is easily inhibited by such conditions as too high a concentration of pyruvate. The first methods for estimating L.D.H. activity measured the amount of lactate formed or pyruvate left. These estimations are not easy and have been replaced by a simple measure of DPNH.

Both DPN and DPNH have a characteristic light absorption peak at 260 mu. DPNH has another peak at 340 mu where absorption by DPN is negligible. This absorption spectrum difference is the key to the most elegant of methods for the estimation of this type of enzyme activity, i.e., the measurement of optical density at 340 mu before and after incubation.

Method: The following reaction mixture is prepared immediately before use:

26.0 ml. of 0.1 molar phosphate buffer, pH 7.8.
1.0 ml. of 0.01 molar sodium pyruvate in phosphate buffer.
3.0 mil. of DPNH, 1.0 mg. per ml., in distilled water.

3.0 ml. of this mixture is put into each cuvette. To one, the control, is added 0.1 ml. of water. To each of the other cuvettes add 0.1 ml. of dilute serum (one part serum and nine parts distilled water). The optical density of each is read immediately, at 340 mu, using the control cuvette to set the spectrophotometer at an optical density of 0.500. All cuvettes are incubated at 37°C for thirty minutes, then the optical density again read in the same way as before. As the optical density of the mixture depends on the amount of DPNH present, and the amount decreases as it is converted to DPN, the optical density decreases. The difference between the two readings for each cuvette, converted into whole numbers, represents the units of L.D.H. activity in that amount of serum, e.g., optical density before incubation is 0.520; optical density after incubation is 0.400. There are 120 units of L.D.H. per 0.01 ml. of serum. The upper limit of normal for this method is 110 units, with an average normal of 90.

A point to consider is that the same cuvette in the same state of cleanliness should be used for the two readings on each mixture. It is better to carry out the whole process in cuvettes than to empty the mixtures into test-tubes for the incubation and risk returning them to the wrong cuvettes, or cuvettes in a cleaner or dirtier state than at the beginning.

Obviously the biggest hurdle to using this method in smaller laboratories is the lack of a suitable spectrophotometer. A simple and reasonably accurate alternative is the colorimetric method developed by L. Berger and D. Broida. Pyruvate reacts with 2, 4, dinitrophenyl-hydrazine to form a "hydrazone" which has a high optical density over a broad wavelength of 400-550 mu. By starting with the same standardised pyruvate substrate which always yields the same "hydrazone", the other components of the mixture contribute very little to the optical density at the chosen wavelength.

GLUTAMIC-OXALACETIC TRANSAMINASE (referred to as SGOT). This enzyme catalyses the transfer of an amino group from the alpha position of aspartic acid to alpha-keto-glutaric acid, forming glutamic and oxalacetic acids. Various methods have been used for measuring this activity.

The accepted method is that of Karmen et al. In the presence of malic dehydrogenase and DPNH, the oxalacetic acid formed is reduced to malic acid, and the DPNH to DPN, the reaction being accompanied by a decrease in optical density as in the L.D.H. method described above. Without rigorous temperature control, this method can give tremendous errors—about 7% for each degree centigrade of variation. Variations in room temperature and the heat of the spectrophotometer itself both contribute to this error.

Tonhazy et al. convert the oxalacetic acid to pyruvic acid with aniline citrate, then estimate the pyruvic acid as its hydrazone. This however is neither sensitive nor specific, as 2, 4, dinitrophenyl-hydrazine reacts with other keto-acids present.

Henley and Pollard modified this method using heat to convert the oxalacetic acid to pyruvic acid which was then converted to lactic acid with L.D.H. and DPN. Such methods, using two separate enzyme systems, with intermediary steps, call for rather more accurate control of reagents and conditions than the average hospital laboratory can manage.

Frankel and Reitman of the Sigma Chemical Company devised a method which is more controllable, relatively simple and reasonably accurate. A substrate of alpha ketoglutaric acid and aspartic acid is incubated for an hour with serum. The oxalacetic acid formed is estimated in alkaline solution as its hydrazone. The colour produced is read at a wavelength of approximately 500 mu. The reagents are reasonably stable and not expensive. This seems to be the best method available for hospital laboratories.

GLUTAMIC-PYRUVIC TRANSAMINASE (referred to as SGPT): This transaminase catalyses the transfer of an amino-group from alanine to alpha-keto glutaric acid, forming glutamic acid and pyruvic acid. This enzyme estimation is often referred to in overseas journals, and may be preferred in assessing liver damage, rather than SGOT.

Methods of estimation are very similar to those for SGOT, and the most suitable is that of Frankel and Reitman.

SUMMARY: Methods of estimation of serum lactic dehydrogenase and serum transaminases are discussed from the point of view of suitability for use in hospital laboratories.

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AN INCIDENT OF FOOD POISONING CAUSED BY HEAT RESISTANT CLOSTRIDIUM WELCHII

B. W. MAIN

(*Pathology Department, Christchurch Hospital.*)

DESCRIPTION OF OUTBREAK

On the 26th September, 1958, members and staff of a local body numbering fourteen in all, made an all day inspection trip of the Board's area, and took with them sixteen boxed lunches prepared by a catering firm which specialised in this type of lunch. The food was left in the cars during a warm morning until lunch was eaten at 1 p.m.

Then to twelve hours later eleven members of the party developed colicky abdominal pain, followed by severe diarrhoea which continued into the following day. Three members of the party were unaffected, but at least one of these did not eat the cold chicken which was a feature of the lunch. However, a member of the Board staff took home the two spare lunches, one of which was eaten that night by his son on his arrival home from University class. Twelve hours later he also suffered the same symptoms.

The Health Department being informed of the incident were able to obtain the other spare lunch which was brought to us in the Pathology Department a few minutes before 5 p.m. the day following the outbreak. Inspection showed a very attractive boxed lunch containing the following:

A leg of chicken and some breast.
Lettuce and tomato.
2 halves of boiled egg.
Ham and egg sandwiches.
2 small meat savoury pies.
A buttered roll and a slab of processed cheese.
2 cakes.

Details of the outbreak were received from the Health Inspector and due to the clinical picture and the nature of the food, Dr. Burns suggested an investigation for Cl. welchii as well as for Staph. aureus and salmonellae-shigellae.

LABORATORY INVESTIGATIONS

Direct smears of the meat items, i.e., chicken, ham and meat pies were made and stained by Gram's method. These were all negative for staphylococci, but in the case of the chicken showed moderate numbers of Gram +ve bacilli resembling Cl. welchii.

Portions of the items were inoculated in Selenite F. medium

and Thioglycollate broth for salmonellae-shigellae and staphylococci respectively. These were negative in all respects.

Further portions of the chicken and other items were inoculated into 4 ml. amounts of nutrient broth in bijou bottles, flamed iron strips added to ensure anaerobiasis and the bottles steamed for one hour.

This treatment kills all vegetative forms and spores of non-heat resistant strains of Cl. welchii, but is insufficient to kill the spores of heat resistant Cl. welchii which will withstand boiling for up to five hours. The cultures were incubated at 37°C overnight, shaken and a generous portion of the broth added to tubes of litmus milk media containing a flamed iron strip and further incubated.

After five hours' incubation a stormy clot was produced in the subculture from the chicken. This was subcultured on to an egg yolk-agar plate containing 100ρ g/ml Neomycin and Cl. welchii antitoxin on one half. A positive Nagler reaction resulted.

Further subculture on a blood agar plate incubated anaerobically for 24 hours produced a pure culture of smooth colonies of Cl. welchii which showed only slight haemolysis which is a feature of heat resistant strains.

None of the other food items showed stormy clot production.

In view of these findings the Health Inspector was requested to obtain faecal specimens from the persons involved but due to most living in the country, and the unco-operative attitude of the remainder who had by now recovered, only four specimens were received.

A bean sized portion of stool was emusified in 4 ml. nutrient broth, a flamed iron strip added, steamed for one hour, and cultured as previously described. Heat resistant Cl. welchii was present in three of the four specimens.

In an effort to trace the source of infection faeces from the staff of the catering establishment were obtained. Two out of the twelve were found to contain the organism. It was elicited in questioning that several members had experienced attacks of abdominal pain and diarrhoea recently. The significance of these findings is not clear as the standard of hygiene in the kitchen was stated to be high. It may be that the staff had themselves eaten chicken at the shop, although this was forbidden by the management.

Going further back still we obtained samples of offal, washing water and whole fowls from the slaughterhouse whence the caterers obtained their supplies. The whole fowls were boiled under normal cooking conditions for 1½ hours, allowed to steep in the liquor at room temperature overnight and portions of fowl and broth

cultured as described. These proved negative as did the other samples.

DISCUSSION

This form of food poisoning has become more prominent in recent years. The incubation period is 8-22 hours, usually 12 hours. Abdominal pain and diarrhoea are the chief symptoms. Nausea and vomiting or pyrexia are rare and the duration is usually 24 hours. In contrast food poisoning due to staphylococcal enterotoxin, produces nausea and vomiting within a few hours of a meal, and salmonella gastro-enteritis produces fever and other signs of infection and has a longer duration of symptoms.

A pure lower bowel infection seems to be produced and heat resistant Cl. welchii can be isolated almost invariably from the stools of those affected while these organisms can be demonstrated in only 5% of normal individuals.

The usual vehicle of infection is meat or poultry which is cooked and eaten cold or after reheating. Taste and smell are usually normal. At some stage in its preparation contamination by Cl. welchii occurs though how this happens is not clear. It may be contaminated by food handlers after cooking, e.g., chicken in many cases is torn to pieces by hand. In any case the spores are not killed by boiling or subsequent reheating. Pressure cooking is the obvious remedy.

In this case we tried to duplicate the preparation of the fowls to the cooked stage as there seemed strong grounds for suspecting the slaughterhouse as the vector of infection. The standard of hygiene was very low indeed but we were unable to isolate the organism.

SUMMARY

An incident of food poisoning due to heat resistant Cl. welchii and methods used to isolate the causative organism is described.

ACKNOWLEDGEMENT

I am indebted to Dr. G. C. T. Burns for help in the preparation of this paper.

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MINUTES OF COUNCIL MEETING OF THE N.Z.A.B.

Held at Wellington on Saturday, April 4, 1959

Present: Messrs Reynolds, Olive, Bloore, Donnell, Walsh, Hutchings, Cameron, Lynch, and Miss Evans.

Minutes of Previous Meeting: Taken as read. Olive-Evans.

Business Arising from Minutes:

Mr Olive reported on the findings of the sub-committee on Standard Biochemical Methods and the action taken.

Moved: That the Council approve the report of the sub-committee on Standard Biochemical Methods. Bloore-Walsh.

Applications and Resignations:

Moved: That Miss R. Allen's application for Senior membership be approved. Olive-Cameron.

The Secretary was instructed to write a letter of apology at the delay in handling her application.

The following new members were elected

Mr L. Cross (Gisborne); Mr P. A. Jones (Tauranga); Mr R. J. Nicholas, Miss J. E. Montgomerie (Palmerston North); Miss D. Norris (Waipukurau); Messrs M. K. Milmine, K. R. James (Hamilton); Miss E. A. Blackie (Westport); Messrs T. E. Brown, A. J. Ansford, and Miss F. E. S. Wright (Timaru); Miss L. Wills (Hawera).

Senior Members: Miss R. E. Allen (Wellington); Miss M. E. Evans (Masterton); Mr D. A. Woodhouse (Hawera). Olive-Hutchings.

The following resignations were accepted with regret:

Mrs R. T. Patten (nee Cantrell); Mrs G. Slyfield (nee Rutherford); Mrs P. A. Smith (nee Waters). Hutchings-Walsh.

Treasurer's Reports

The Treasurer reported on the current financial position.

Moved: That the expenses of the Council meeting be approved. Walsh-Olive.

Moved: That the Hon. Secretary be authorised to act as Treasurer until the next election of officers. Olive-Bloore.

Moved: A vote of thanks to the retiring Treasurer. Reynolds-Olive.

Journal Report:

After discussion it was proposed that the July Journal be delayed until August for Conference news.

The Editor reported that addressograph plates had been ordered.

The question of overdue subscriptions was discussed briefly in its application to the Journal.

Moved: That the matter of overdue subscriptions be considered at the next Council meeting. Reynolds-Hutchings.

Moved: That the Editor's report be accepted and the Editorial Committee be thanked for its work. Bloore-Olive.

Correspondence:

(*a*) Rejection of application for membership of Canterbury Medical Library.

The matter of the library was referred to Conference 1959.

(*b*) Affiliation of Auckland Group.

The Secretary was instructed to circulate the proposed Rules of the Branch to Council members.

(*c*) Name of Association.

Mr Olive suggested that several alternatives be presented to Conference and that delegates be asked whether a postal ballot should be held on these proposals or a decision made at the Annual General Meeting.

Moved: That the matter of the name of the Association be referred to the next Council meeting. Olive-Lynch.

The Secretary was instructed to write to the Secretary, I.M.L.T. to enquire about Mr Norman's visit.

(*d*) Salaries Advisory Committee.

Moved: That nominations for membership remain the same. Hutchings-Bloore.

Submissions:

1. That double time rates apply from 12 midnight to 8 a.m. as submitted in November 1955 and in June 1957.

2. That the salary range of Grade Laboratory Officers be reviewed with the object of increasing the range and bringing salaries into line with those of the Public Service.

Note: Whereas current P.S. advertisements indicate the possibility of a Scientific Officer achieving a salary of £1850 p.a. there is no possibility of any officer graded by the Hospital Officers Grading Committee receiving more than £1640 p.a. Olive-Hutchings.

Examinations:

The Secretary indicated the proposals and suggestions received in reply to his notice.

The President told the meeting of the action he had taken in writing to the Hospital Laboratory Advisory Committee about examinations.

The Secretary read associated correspondence with the Health Department suggesting an approach to the Society of Pathologists. The Secretary was instructed to write to the Secretary of the Society saying that the Association did not, at present, wish to approach the pathologists directly, but that should any committee be formed to consider the examinations the Association would like to be represented. Copies to the Director-General and Secretary, Hospital Laboratory Advisory Committee.

International Association of Medical Laboratory Technologists:

The Secretary was instructed to circulate the material received from the International Association to Council members.

Moved: That Inward correspondence be received Olive-Walsh.

Moved: That Outward correspondence be approved. Olive-Bloore.

General Business:

Mr Donnell spoke of the need to formulate concrete attitudes for the Association regarding the examinations. He was supported strongly by Mr Hutchings. After brief comment by Mr Reynolds and Mr Olive the matter was referred to the next Council meeting.

The meeting closed at 3.40 p.m.

MINUTES OF A COUNCIL MEETING OF THE N.Z.A.B.

Held at Invercargill on July 1, 1959

Present: Messrs Reynolds, Olive, Bloore, Donnell, Hutchings, Cameron, Lynch and Miss Evans.

Apology: Mr Walsh.

Mr Thompson, of Invercargill, Conference organiser, attended by invitation and reported on arrangements for Conference.

Minutes of Previous Meeting:

Moved: That these be taken as read. Olive-Hutchings.

Business Arising from Minutes:

Moved: That the Treasurer be instructed to make receipts in triplicate one copy for file, one copy to be sent to payee, and one copy to the Editor. Olive-Hutchings.

Affiliation of Auckland Branch:

After discussing points in the Rules of the Branch as circulated to members it was moved:

That the affiliation of the Auckland Branch be approved and the Rules accepted provided that: Clause 2 be deleted; and in Clause 4 and elsewhere where applicable the words "head Association" be amended to "parent body"; and in Clause 8(a) the word "subscription" be amended to "branch levy". Olive-Bloore.

Change of name of Association:

Moved: That the suggestions regarding change of name of the Association go forward to the Annual General Meeting for approval and that on approval a postal ballot be conducted and the Council implement the finding of that ballot. Hutchings-Olive.

The names suggested were:

Medical Laboratory Technologists, Medical Analysts, Pathology Technologists, Medical Laboratory Practitioners.

International Association of M.L.T.:

Moved: That the matter of the I.A.M.L.T. be deferred until the question of the name of the Association is determined. Olive-Cameron.

Examinations

Moved: That the matter be referred to the incoming Council after hearing the opinion of members as expressed at the forum at Conference. Olive-Hutchings.

Moved: That the Minutes be approved. Hutchings-Evans.

Applications and Resignations:

Moved: That the applications for membership as approved by postal ballot be accepted and the resignations be accepted with regret. Olive-Evans.

Moved: That a letter be sent to Miss Hitchcock wishing her a speedy recovery and that she be notified that she is financial for the period of her illness. Olive-Lynch.

Balance Sheet: The Balance Sheet was presented by the Hon. Secretary.

Journal Report: The Editor reported on Journal matters and the Essay Competition.

Moved: That the Editor of the Journal be authorised to establish a bank account for the Journal of the N.Z. Association of Bacteriologists (Inc.). Donnell-Cameron.

Moved: That the expenses of the meeting be approved. Olive-Lynch.

The meeting closed at 10.20 p.m.

MINUTES OF COUNCIL MEETING HELD ON JULY 3, 1959

Present: The whole Council.

Moved: That Desmond James Philip, Malcolm McLeod Donnell and Graeme Lindsay Cameron be authorised to operate the bank accounts of Association. Reynolds-Hutchings.

MINUTES OF THE FIFTEENTH ANNUAL GENERAL MEETING OF THE N.Z. ASSOCIATION OF BACTERIOLOGISTS (INC.)

Held at Kew Hospital, Invercargill, on July 2, 1959

Mr J. A. Cushen, Chairman of the Southland Hospital Board, extended a hearty welcome to delegates and wished all well for a profitable Conference.

Dr. H. Hunter, Medical Superintendent, in his remarks, said that he thought no ancillary service was leaned on by the medical staff more than the laboratory. He welcomed delegates and declared the Conference open.

Dr. N. G. Prentice, Pathologist, spoke to delegates and criticised the Conference organisation and commented on the need for changing the name of the Association.

The President thanked him and said that most of the points of criticism were already receiving the attention of the Council.

Roll Call: The following attended the Conference.

Mr D. W. Fitzgerald (Timaru); Messrs G. Tait, M. Lynch, S. W. Josland, D. G. Till, H. T. G. Olive, L. Reynolds, Misses A. J. White, B. Scully, S. Harding (Wellington); Messrs S. C. Marshall, W. Poole (Wallaceville); Miss J. Cater, Mr A. F. Harper (Wanganui); Mr G. D. C. Meads (New Plymouth), Mr K. G. Reeve (Dannevirke); G. R. George (Rotorua); D. C. Smith (Tauranga); I. R. Buxton (Oamaru); H. G. Bloore (Blenheim), M. O. Ekdahl (Gisborne); G. Lyon (Lower Hutt); G. W. McKinley (Waipukurau); R. Barrington (Hawera); L. R. Wilson, F. C. Kershaw, J. D. R. Morgan, Misses A. Butcher and A. Ford (Dunedin); M. G. Harper, C. K. Clapson (Dunedin), J. C. Mann, H. E. Hutchings (Palmerston North); J. W. Carroll (Hastings); D. J. Dunlop (Napier); M. R. Morris (Clyde); N. E. Jarman, M. McCarthy, K. A. G. Watts, D. J. Philip, W. J. Sloan, G. L. Cameron, F. M. Rush-Munro, M.McL. Donnell (Auckland); H. E. Foster (Ashburton); N. D. Johnston, K. B. Ronald (Whangarei); Miss M. E. Evans (Masterton); Miss C. Saxby (Napier); R. W. Smail, G. C. Thompson, Misses J. Gray and C. McKenzie (Invercargill); Misses G. Collyer, P. Scarf, C. B. Curtis, L. Evans, Messrs B. Main, D. H. Adamson (Christchurch); Miss E. Blackie (Buller),; Miss E. Withy (Bay of Plenty); M. G. Sutherland (Nelson); Miss H. MacDiarmid (Ruakura).

Apologies were received from the following:

Miss J. Mattirgley (Wellington); Mr F. J. Austin (Dunedin); Mr J. J. G. Peddie (Upper Hutt), Mr Buxton, Senr. (Wanganui); Messrs R. T. Kennedy, P. H. Curtis, K. M. Bilkey, V. Jones, D. Taylor, C. Masters, E. Eves, R. Stockwell, Misses M. Lindsay, H. Chesterman, B. Pearce, R. McLeay, Mesdames L. Nijenkamp-Beltman, P. McKain (Auckland).

President's Address:

Mr Reynolds reviewed Council activities of the year and then spoke briefly of the future. He spoke of the need for review of examinations and training and said that the forum to be held at the Conference would provide an opportunity for members to give Council some idea of their feelings in these matters. He referred to the matter of changing the name of the Association and stressed the need for unity, especially with the trend to specialisation in branches of medical laboratory work. In closing, Mr Reynolds mentioned briefly the visit of Mr E. Norman, M.B.E., a founder of the I.M.L.T. and that Council hoped to have discussions with him.

Minutes of the Fourteenth Annual General Meeting:

Moved that these be taken as published. Olive-Foster.

Business Arising from Minutes:

Mr Olive reported on action taken concerning Standard Biochemical methods.

Change of Name of Association:

The Secretary indicated the names suggested to Council.

Mr Hutchings recommended the name Institute of Medical Laboratory Technology.

Mr Bloore suggested the name Clinical Laboratory.

Miss Curtis suggested Medical Laboratory Sciences.

After further discussion it was moved:

That a postal ballot be held of the membership of the Association as to whether the name of the Association should be changed.

Olive-Main.

After the luncheon adjournment Mr Bloore proposed a motion concerning the form of the ballot. Some criticism of the wording of the motion caused Mr Bloore to reform it as follows

That the postal ballot take the form: (a) Is a change of name desired; (b) Which of the following recommendations do you favour should a change of name be approved. Bloore-George.

Mr George said that he thought there was only one choice; that was to follow international practice and use the term Medical Laboratory Technology. After a ballot on show of hands the following motion was carried.

That the two names Medical Laboratory Technology and Clinical Laboratory Technology be submitted to ballot as the recommendations of this meeting. Hutchings-Till.

Mr McKinley asked what action Council would take following the ballot.

The President said that a Special General Meeting would be called associated with a Council Meeting.

Moved: That the Minutes as published be approved.

Kershaw-Morgan.

FOURTEENTH ANNUAL REPORT, JULY, 1959

Ladies and Gentlemen,

I have the honour to present the Fourteenth Annual Report of the New Zealand Association of Bacteriologists (Inc.).

Membership now stands at 189 senior members, 214 junior members, 16 honorary members and 4 life members. There have been 9 resignations and 33 new members elected.

Finance: The Balance Sheet will be presented separately. Mr Walsh retired from the Treasurer's position during the year. The Association is greatly indebted to him for his sterling work over the past few years.

Conference 1958: Wellington acted as hosts for an enjoyable Conference last year. Our thanks are due to the Conference Committee for their effort.

Proceedings of Council: The Council met in April this year when the first application to become an affiliated branch was received from Auckland as allowed for in the new Rule adopted last year.

Consideration has been given to the proposed change of name of the Association and the result of these deliberations will be considered at this meeting later.

Submissions have been made to the Salaries Advisory Committee to seek adjustment of some discrepancies.

During the year the opinion of members was sought about examinations. The circulated notice about this met with very poor response. Some good suggestions were received and are under consideration by Council. The general subject of training and examinations will be discussed during this Conference.

Further correspondence has been received from the International Association of Medical Laboratory Technologists and a report on these matters will be made to this meeting.

Journal: The Journal has had a rather better year this year for its early issues but the supply of material is running short again. Members are urged to maintain their efforts and so ease the not inconsiderable task of the Editorial Committee to whom we owe much gratitude.

Essay Competition: This is now being conducted by the Journal Committee and the Editor will report on the results of the competition.

It is good to be breaking new ground in being the guests of one of the smaller centres for this Conference. The Association appreciates the hospitality extended to its members by Invercargill. The Council offers its good wishes to you all for a successful and informative Conference.

For and on behalf of the Council,

M. McL. DONNELL,
Hon. Secretary.

Moved: That the Annual Report be approved. Donnell-Meads.

Essay Competition: The two winners were:

Technical Section: Mr M. Lynch, Wellington.

Essay Section: Miss R. Briant, Gisborne.

Moved: That a hearty vote of thanks be accorded the Editors for their work. Olive-Donnell.

Balance Sheet: The Secretary presented the Balance Sheet.

Moved: That the Balance Sheet be adopted. Donnell-Olive.

ELECTION OF OFFICERS FOR 1959-60:

The following officers were elected:

President: Mr L. Reynolds (Wellington).

Vice-Presidents: Mr H. T. G. Olive (Wellington), Mr H. G. Bloore (Blenheim).

Secretary: Mr H. E. Hutchings (Palmerston North).

Treasurer: Mr D. J. Philip (Auckland).

Council Members Miss L. Evans (Christchurch), Mr M. McL. Donnell (Auckland), Mr G. Cameron (Auckland), Mr M. Lynch (Wellington), junior member.

Members expressed their appreciation of the work of the retiring Secretary and Treasurer by acclammation.

Moved: That the ballot papers be destroyed. Morgan-Kershaw.

General Business:

International Association of Medical Laboratory Technologists:

The President informed delegates of correspondence with the International Association.

Remit re T.B. Compensation:

Mr Main spoke on behalf of the remit.

The Secretary referred to correspondence with the Department of Health in the matter.

The President said that there appeared to be some change in attitude by the Department.

The remit was put to the meeting and carried.

Moved: That the Council of the Association make representation to the Director-General of Health regarding the universal witholding of approval for a Hospital Board to continue payment of full salary to a Laboratory Worker after a period of three months when he contracts Tuberculosis in the course of his employment.

The object of this representation being: That where the Pathologist or Medical Officer in Charge is of the opinion that the disease has been contracted in the course of employment, full salary for the period of incapacity should be automatically approved. Main-Foster.

Remit that the Editor should be a Council Member:

After discussion the remit was withdrawn and the following Motion substituted.

Moved: That the Editor of the Journal be invited to all Council Meetings if he is not a Council Member. Main-Till.

Bank Account for the Journal:

Moved: That the Editorial Committee be empowered to open an account at a trading bank in the centre of publishing for the purposes of the business of the Journal of the N.Z. Association of Bacteriologists and that an audited annual statement of accounts be presented to the Treasurer and that initially a sum of One Hundred Pounds (£100) be deposited from the Association funds with the Editorial Committee. Olive-Ronald.

Moved: That the Honoraria remain the same and be paid Olive-McKinley.

Some general discussion ensued on the subject of Gradings.

Mr Bloore enquired if anything was known of the position regarding the staffing of laboratories in Auckland with laboratory assistants and the fact that Intermediate trainees had not been accepted there for completion of training.

Mr Philip spoke briefly of what little he knew of the matter.

Mr Bloore proposed a Motion which he withdrew after the tea adjournment and after further discussion of the matter the following Motion was put to the meeting and carried.

Moved: That a protest be sent to the Director-General of Health from this meeting deprecating the circumstances giving rise to the situation where trainees who have passed the Intermediate Examination are unable to obtain positions on a pathologist controlled laboratory to complete their training. Bloore-Olive.

Conference 1960:

An invitation was received and accepted from Christchurch.

Moved: A vote of thanks to the Chair. McKinley-Olive.

The meeting closed at 8 p.m.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

February, 1959

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Drs. F. B. Desmond, G. C. T. Burns, S. E. Williams.

WRITTEN EXAMINATION

Tuesday, February 24, 1959

(Time allowed: 3 hours. Six questions, all to be attempted.)

1. Describe the life cycle of Echinococcus granulosus (taenia echinococcus) as it occurs in New Zealand. State what you know of any two tests used to demonstrate human infection with this parasite.
2. Discuss protein precipitants used in quantitative biochemical analysis, stating the advantages and disadvantages of each.
3. Discuss the routine laboratory tests likely to be requested in an adequate *preliminary* investigation of a patient with an abnormal bleeding tendency. Describe in detail any two of the tests.
4. Give an account of the laboratory diagnosis of Clostridial infections.
5. Discuss methods of controlling the accuracy of tests performed in a routine clinical biochemical laboratory.
6. Write short notes on:—
   (a) Heterophile antibodies.
   (b) E.S.R.
   (c) Cold agglutinins.
   (d) Phenotype.
   (e) Histological tissue fixation.

PRACTICAL I

Tuesday, February 24, 1959

Time allowed: 3 hours.

BIOCHEMISTRY

(First section)

1. Perform (a) colloidal gold reaction;
   (b) a thymol turbidity test;
   on the serum specimen G.
2. Determine the calcium concentration of specimen H by the method of Clarke and Collip and use overnight precipitation.

These three estimations to be completed on Wednesday afternoon.

3. Estimate the CO_2 combining power of specimen I.

BACTERIOLOGY

(First section)

1. Identify culture A as far as possible in the time allowed.
   (*S. typhi murium.*)
2. Specimen B is a swab of pus. Identify any organisms that it contains.
   (*Pneumococci* and *Staphylococci.*)
3. Identify culture C, as far as possible. What further investigations would you suggest?
   (*C. diphtheriae.*)

Questions 1, 2 and 3 to be completed on Wednesday morning.

4. Identify cultures D and E, as far as possible. What further investigations are indicated?
   (*D—Cl. welchii.*)
   (*E—Cryptococcus neoformans.*)

PRACTICAL II

Wednesday, February 25, 1959
Time allowed: 3 hours.

BACTERIOLOGY

(Second section.)

1. Complete questions 1, 2 and 3 from previous day.
2. Stain and examine the fixed film F for acid fast bacilli.

HAEMATOLOGY

(First section)

1. Specimen K is serum from an Rh (D) Negative woman collected during the thirty-sixth week of her third pregnancy.
   Determine the titre of Rh (D) antibodies in saline and albumin. (*Saline titre; 1: 16—Albumin titre; 1: 32.*)
2. (a) Perform an ABO and Rh (D) grouping on specimen L.
   (b) Using saline and Coombs techniques, cross-match L with serum M. (*Compatible.*)

PRACTICAL III

Wednesday, February 25, 1959
Time allowed: 3 hours.

BIOCHEMISTRY

(Second section.)

1. Complete questions 1 (a) and (b) from previous day.
2. Complete question 2 from previous day. Give your answer in both mg. per 100 ml. and in milliequivalents per litre.
3. Determine the normality of the sodium hydroxide specimen J. What is a normal solution?

HAEMATOLOGY

(Second section.)

1. Do an absolute reticulocyte count using a dry slide technique on specimen N.
2. Examine and report on blood films O, P, Q. R. S. T.
   O—Erythroblastosis foetalis.
   P—Neutrophil leucocytosis.
   Q—Hypochromic anaemia.
   R—Pernicious anaemia.
   S—Acute leukaemia.
   T—Glandular fever.

   Write notes on the following spots:—
   1. Selenium cell.
   2. Calcium chloride.
   3. Cystine crystals in urine.
   4. Pfeiffer in C.S.F.
   5. Asbestos bodies.
   6. Plasmodium falciparum.
   7. Trichuris ova.
   8. Adult Taenia saginata.
   9. Ascaris lumbricoides.
   10. Vincents organisms.
   11. Section stained with H & E (Section was not properly cleared).
   12. Electrophoretic strip.

The candidates were asked about the following in the oral examinations:

Dr. Williams: T.B. media, T.B. sensitivity tests, Differentiation of human and bovine T.B. Guinea pig post-portem appearances in T.B. and Brucellosis. Meningitis. Methods of sterilization.

Dr. Desmond: Standard controls in biochemistry.

Dr. Burns: Food poisoning, sterilization of media, Haemophilus X and V factors. Milk testing, "Satellitism."

SUCCESSFUL CANDIDATES:

Miss J. E. KIRKNER, Wellington Hospital.
Miss S. M. HARDING, Wellington Hospital.
Miss M. I. EDWARDS, Wellington Hospital.
Miss J. A. STYLES, Christchurch Hospital.
Mr T. E. TANNER, Christchurch Hospital.
Miss P. J. PRENTICE, New Plymouth Hospital.
Mr T. E. MILLER, New Plymouth Hospital.
Miss Y. A. CLARKE, Auckland Hospital.
Mr R. DOUGLAS, Auckland Hospital.
Mr G. J. HILL, Auckland Hospital.
Miss J. E. HOLLOWAY, Auckland Hospital.
Mr M. D. McCARTHY, Auckland Hospital.
Mr A. D. NIXON, Auckland Hospital.
Mr B. J. YEARBURY, Auckland Hospital.
Mr M. R. DIX, Auckland Hospital.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

April, 1959

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Dr. G. E. Fairbrother, Dr. J. D. Reid, Mr G. R. George, Mr N. J. Ellison.

WRITTEN EXAMINATION

Tuesday, April 28, 1959.

Time allowed: 3 hours.

1. (a) You are asked to examine a throat swab for K.L.B. Describe how you would carry out the investigation and positively identify this organism.
   (b) Describe briefly the composition and preparation of Loeffler's medium and Tellurite medium. Why are these used for the isolation of C. diphtheriae.
2. (a) Give a brief account of the main factors influencing the growth of bacteria.
   (b) Describe the main principles of colorimetry.
3. (a) Illustrate by a table or diagram the interactions of the sera and cells of all the A B O blood groups.
   (b) Assuming the A B O and D blood groupings to have been adequately performed, detail your technique for a cross match with a patient known to have had previous transfusions.
4. Discuss the chemical principles underlying the various steps in estimation of blood sugar.
5. (a) Describe briefly the procedure you follow when working with tuberculous material to ensure your personal protection and that of your co-workers.

(b) Write short notes on (1) Bacteriostasis, and (2) Anaphylaxis.
(c) Outline the development of a neutrophil polymorphonuclear cell. Indicate where in the body the various stages are normally present.
(d) Define a normal solution. What is the composition of a normal solution of saline and of normal saline as is used in hospital

PRACTICAL EXAMINATION I

BACTERIOLOGY

1. (a) Titre the serum provided for Br. abortus.
(b) The culture provided was from a dysentery stool. Identify the causative organism, explaining the steps you have taken.
(To be completed next day.)
(*Organism was S. sonnei.*)
(c) Stain the sputum smear provided by the Ziehl-Neilson method and report on it.
(*Tubercle bacilli present.*)
2. Examine the thoat swab provided. Culture and perform sensitivity test.
(*Organism was a Staphylococcus.*)

BIOCHEMISTRY

1. Estimate the total and free acid in the specimen of gastric juice supplied.
2. Estimate the chlorides in the given sample of C.S.F.

PRACTICAL EXAMINATION II

BACTERIOLOGY

1. Complete bacteriology from yesterday.
2. Examine the urine supplied for:
Albumin (*present*).
Sugar (*present*).
Acetone (*present*).
Examine the centrifuged deposit and report on the organisms present.
(*Staphylococcus and coliform bacillus.*)

HAEMATOLOGY

1. Determine the A B O group of the two washed cell suspensions 'X' and 'Y'. Briefly state the steps in your technique.
2. Perform a red cell count and leucocyte count on the blood specimen provided. Show how the leucocyte count is calculated. Make you own films and stain with Leishman. Comment on the film. No differential required.
(*Normal blood.*)
3. Examine and report on the films supplied. State what clinical condition they could indicate. No differential count required.
1. *Hypochromic anaemia.*
2. *Myelogenous leukaemia (chronic).*
3. *Glandular fever.*
4. *High leucocyte count from a patient with an acute infection.*
4. Assuming a given haemoglobin of 11 g. per 100 ml.; use the haematocrit tube supplied and calculate the M.C.H.C. Show your working and state the normal range. What is your comment on the results Briefly outline the essential steps to obtain the P.C.V.

The following subjects were discussed in the Oral examination:— ABO erythroblastosis, ABO incompatibility, Coombs Test, Treatment of phenol burns, Anaerobic media, Differentiation between pathogenic and

saprophytic acid fast bacilli, T.B. media, pH, Free acid in gastric secretions, Indicators, C.S.F. chlorides, Physiological and normal saline, Objectives, Magnification of microscopes, Red cells and yeast cells in urine, Koch, Pasteur, Centrifuges, Numerical aperture, Microns, K.L.B. and inoculation of guinea pigs, MacConkey's agar, Sterilisation of media containing serum, Leishman's stain, Methylene blue, Water testing.

SUCCESSFUL CANDIDATES:

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Miss L. A. MORTLEMAN, Wellington Hospital.
Miss M. B. SCULLY, Wellington Hospital.
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Miss P. M. SCARF, Christchurch Hospital.
Miss B. M. PEARCE, Auckland (Medical Laboratory).
Miss H. F. BROMLEY, Palmerston North Hospital.
Mr K. B. ASH, Palmerston North Hospital.

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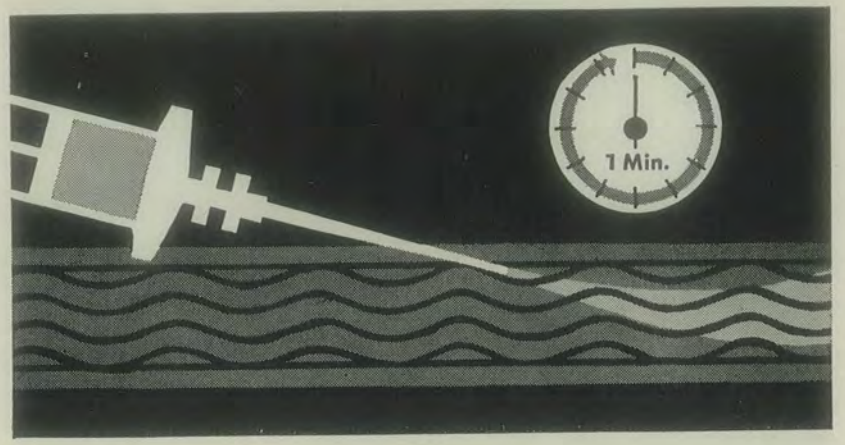

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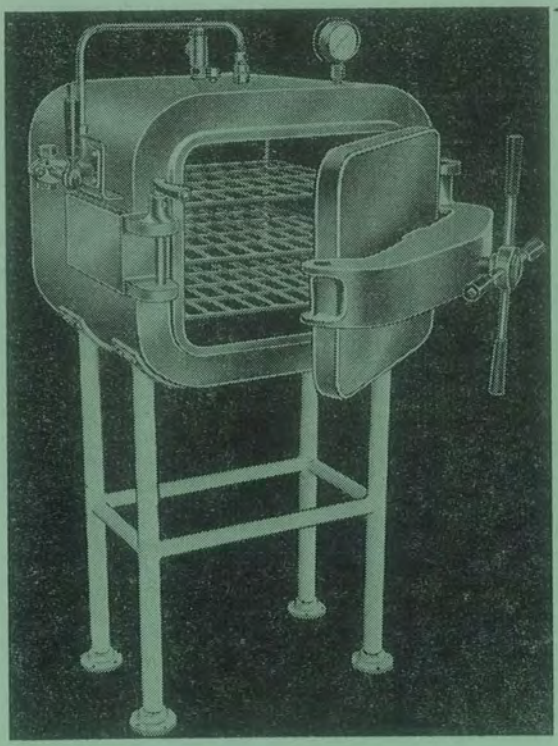

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