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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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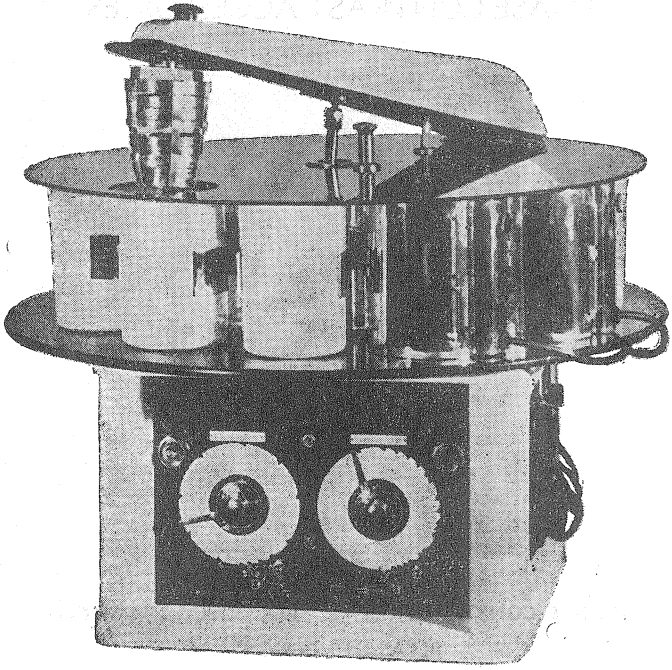

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JOURNAL
of the
NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

Vol. 8, No. 3. October, 1953

EDITORIAL

Recorded in the minutes of the 9th Annual Conference held recently in Christchurch, is the report of a meeting between the Executive of the Pathological Society and representatives of the Association of Bacteriologists. A number of points were discussed but few concrete decisions resulted. It is interesting to note that the Pathological Society does not favour the appointment of a Senior Bacteriologist on the panel of examiners. No doubt there are adequate reasons for this decision and it is not intended to discuss the matter here. What is more interesting however, is the fact that the incoming Executive of the Pathological Society is to review the content and organisation of the final qualifying examination.

As at present constituted, the final examination could hardly be more unsatisfactory. The general scope and method of conducting the examination has not altered significantly in the last 20 years. During this time Hospital Laboratory work has changed out of all recognition and it is therefore to be hoped that the Executive of the Pathological Society will not be too conservative in its approach to the problem and that something more representative of modern laboratory work will emerge.

With the vast increase in Hospital Laboratory work in the last 10 years the syllabus has become too large and unwieldly making it impossible for candidates to have anything but a superficial knowledge of the many subjects involved. To make matters worse there is never any choice of questions allowed in the theoretical paper. It would seem that sooner or later the final examination will have to be split into two or more examinations, with clearly defined syllabuses. This would be of advantage to both candidates and examiners. At present the final examination is largely a repetition of the intermediate one, and while Bacteriologists may hesitate to alter the form of the latter so soon after its inception, it may be necessary to limit its scope by confining the syllabus to say—media making, solutions, histological technique, and elementary haematology; leaving bacteriology biochemistry, and advanced haematology, to the final examination.

Likewise the organisation of the examinations will have to be changed. In the near future it will not be possible to muster all candidates in one place and examinations will have to be held in their own laboratories or in the main centres.

The reorganisation of the examination will not be an easy matter. Let us hope that something more than a slight modification of the present system will eventuate.

PHYSIOLOGICAL ASPECTS OF SOME BACTERIA ISOLATED FROM CLOVER ROOTS

Melva N. Crozier.

Canterbury Agricultural College, Lincoln.

This survey briefly covers investigations which were begun with the intention of studying some physiological aspects of Rhizobium trifolii, the root nodule bacterium. This organism is of importance because it is the symbiotic nitrogen-fixing bacterium associated with clover roots, in this case, those of subterranean clover. While fulfilling its object to some extent, the investigation has indicated also an interesting new field of study—a field which only recently has occupied the attention of some few workers.

Before I elaborate further on this latter finding, I should like to draw a brief historical background to the subject of Rhizobium and to give some reasons for my interest in the root nodule bacterium.

Since Beijerinck in 1888 first isolated this bacterium from leguminous roots, the genus *Rhizobium* has been a favourite object of study for students of agricultural bacteriology. From earliest times the agricultural importance of the Leguminosae has been recognized. The Greek philosopher Theophrastus, 370-285 B.C., in his "Enquiry into Plants" spoke of leguminous plants as "re-invigorating" the soil. He said "Beans . . . are not a burdensome crop to the ground; they even seem to manure it, because the plant is of loose growth and rots easily; wherefore the people of Macedonia and Thessaly turn over the ground when it is in flower.' The reason for this invigorating effect of legumes on the soil remained a mystery right up to comparatively recent times when Hellriegel and Wilfarth (1888) proved beyond doubt that root nodules are responsible for the peculiar ability of leguminous plants to use atmospheric nitrogen.

The thousands of papers which have appeared since 1888, describing the habits and characteristics of the nodule bacterium from the bacteriological, the chemical, the agricultural and the agronomical point of view, indicate the prime significance of this bacterium and its legume host. Fred, Baldwin and McCoy (1932) epitomised the reason for the interest of so many workers in the symbiotic relationship of plant and bacterium when they stated. . . . "It is no exaggeration to say that a comprehension of the root nodule bacteria and their association with the Leguminosae would disclose to the world the basic principle of soil fertility."

Like the majority of bacterial types, the root nodule organism has undergone change of name, and has been recorded under such genera as *Bacillus*, *Bacterium*, *Pseudomonas* and *Rhizobium*. In view of the difficulties associated with bacterial classification, it is not surprising that Beijerinck's *Bacillus radiciola* should have suffered frequent change of taxonomic status until finally settled by Frank in the now generally accepted genus of *Rhizobium*.

Early in the study of *Rhizobium*, it was found that certain strains were effective in inducing the formation of nodules on some genera of the Leguminosae, while on others they apparently lacked this ability. Nobbe and Hiltner (1896) claimed to have shown differences among *Rhizobium species* . . ." It has been established by us an entirely new fact that the tubercle bacteria of the various Papilionaceae are of full strength (i.e., in the production of efficient nodules or tubercles) only with that species from whose root tubercles they were themselves obtained. With nearly-allied species they are of weaker strength, and with systematically different species they are useless."

Accordingly, if an organism from the nodule of a leguminous plant was found to effect nodulation on another plant, the two plants were considered to belong to the same "cross-inoculation group." Since Fred, Baldwin and McCoy (1932) published a list of sixteen of these groups, suggestions have been made that certain groups should be combined and others added, until more than twenty such groups have been proposed. Even this number does not entirely satisfy those workers who may be classed as taxonomic "splitters." Contrary to these views, taxonomic "lumpers" have aroused considerable doubt concerning the validity of cross-inoculation groups. Wilson (1939) concluded from his review of the results of various workers, as well as from his own work, that neither a sufficient number of plant species nor a sufficient number of diverse strains of the organism had been employed by investigators to justify the establishment of definite cross-inoculation groups or to encourage their use. He suggested that though the groups may be useful in a practical way, certainly from the scientific standpoint they should be abandoned. This conclusion and suggestion resulted from a study of the nodulating performances of isolates from numerous legumes and from a study of the morphology and physiology of such isolates.

In New Zealand, where agricultural improvement is vital to the economy of the country, the importance of a legume component in pastures of high productivity can hardly be over-emphasized. The increasing areas of land yearly being brought into cultivation calls for the practice of scientific agriculture and the use of especially suitable plant cover. In many cases this must be of such a type that it has the ability to establish itself on light soils and under conditions unfavourable to the successful growth of the plant types commonly employed. A legume possessing these attributes is found in subterranean clover which, by virtue of its ability to survive in areas of such low fertility that the usual white clover—ryegrass association is unsuccessful, is becoming increasingly popular for use in the improvement of light land and the development of high country pastures.

It becomes important, then, to have some knowledge of the strain-of bacterium associated with *Trifolium subterraneum*, and to determine whether nodulation may be affected by any of the types of *Rhizobium* usually present in the soil or only by a specific strain of *Rhizobium trifolii*. If this latter is the case, then it may be necessary to artificially

inoculate subterranean clover seed with its specific strain of nodule-forming bacterium. This is the method commonly used with lucerne seed. In this respect also, it is important to possess some knowledge of the incidence and "effective levels" of *Rhizobium trifolii* in different soil types and in different localities.

This investigation, therefore, was begun with the idea of studying the nodule bacterium associated with subterranean clover. Its incidence in different soil types was put under preliminary investigation, and an attempt was made to learn some of the physiological characteristics of the bacterium itself. These proposed studies, however, were diverted into other channels when it was found that bacterial types other than *Rhizobium* were being studied. These types, although in many cases similar to *Rhizobium* in microscopic appearance, all differed from it in their biochemical reactions. But I shall start from the beginning.

With a good deal of relevant literature behind me, a large amount of perhaps misplaced enthusiasm and with only a theoretical knowledge of the elements of bacteriology, I began a study of *Rhizobium trifolii*

Right from the start my isolates gave biochemical reactions which often differed from those as described in the literature. To further complicate matters, records of the biochemical activities of *Rhizobium* can only be described as highly contradictory.

I must mention also, the extreme pleomorphism exhibited by the root nodule bacteria. It has been shown by various workers that *Rhizobium* may exist in as many as five different life forms: a non-motile pre-swarmer stage, a larger non-motile pre-swarmer stage, an actively motile swarmer, a motile rod stage, and a stage of high vacuolation. It is reported that all these variations may be obtained in the laboratory by altering conditions and the nutrients supplied to the bacterium. However, the odd X and Y shapes known as bacterioids in which form *Rhizobium* occurs in the actively functioning nodule, always revert to the rod from when cultured on artificial media.

Microscopic examination, therefore, reveals such numbers of forms that the beginner at any rate is thrown into a state of utter confusion. As far as I can determine, even the experts accept the capacity to produce nodules as the only true criterion of the identity of Rhizobium.

For six months I re-isolated, subcultured, tested and repeated, until I was forced to admit that there were bacterial types other than Rhizobium in my cultures, and as far as I was aware, they were not there through any fault in my isolation technique.

This conclusion led to an investigation into the efficacy of the usual methods of sterilization of the nodule surface. I might explain here that to isolate Rhizobium from small nodules, the nodule is removed from the root, surface sterilized, usually with alcohol and mercuric chloride, washed in sterile water and crushed aseptically. Larger nodules, e.g., those of peas or beans, are surface sterilised in the same

way, then cut open and a small portion of the inner contents removed. To test the efficacy of sterilisation four different methods were followed, after which the sterilised but uncrushed nodules were agitated in a liquid medium for a short time. The resultant broth cultures were streaked out after suitable periods of incubation. In this way, it was found that very often bacteria could be cultured from the sterilised nodule surface, and these obviously would be cultured with the nodule-crushing in the process of isolation.

While the variations from the standards which I had found to occur in my cultures could all be explained by the presence of contaminants from the external nodule surface, there appeared to be another possibility—that different bacterial types inhabited the nodule with *Rhizobium*. This idea is not new, in fact various workers since 1888 have reported a number of different bacterial types as having been isolated from the nodule interior, but such reports have always been treated with suspicion because of the possibility of their being contaminants from the outer nodule surface. Subterranean clover nodules are too small for convenient study of their nodule contents, but it was reasoned that as the nodule is an outgrowth of the cortical tissues of the root, it would appear likely that bacteria already existing in the tissue may become incorporated into the new structure and there continue to exist in some sort of "passive" association with the plant. This concept of a bacterial flora in healthy plant tissue is a comparatively new one, so you may be interested if I briefly survey the pertinent literature. The older concept . . . glasshouse conditions.

The older concept that healthy plant tissue is free of microbial life appears to date from Pasteur (1876) who reported that the juice of grapes when expressed aseptically showed no sign of fermentation when maintained under sterile conditions. Berthold (1917) after reviewnig the literature, was another who claimed the aseptic nature of normal plant tissues.

One of the first investigators to seriously challenge this generally accepted view was Perotti (1926) who isolated bacteria from the roots of many families of plants, and from his studies claimed the usefulness of the concept of the "bacteriorhiza" a term originally introduced by Hiltner (1904) and which, subject to the modification of various workers, has proved a most useful basis for further investigations. Perotti reported bacteria within the root diffused in the cortex, sometimes in the outermost zone of the phloem, in intercellular spaces and even in the interior of cells. Such "infection," he believed, was limited to a well-defined region, viz., the cortex and outermost phloem. He postulated that the forms constituting this "bacteriorhiza," or union of plant and bacteria, were not a single fixed species but rather an assemblage of physiological types. To Perotti, then, may be accredited the view that the healthy root surface is not the limit of bacterial penetration and that there exists a modified population in a well-defined zone in the root tissue. Beyond this zone, according to Perotti, no form of micro-

organism is traceable and it is here that is begun "the biophysical and biochemical evolution of useful and useless products of their cumulative metabolism."

Hennig and Villforth (1940) upheld the findings of Perotti when they studied 28 different healthy plants and found bacteria (mostly spore-formers) in all parts (leaves, twigs, stems and roots) of all the plants studied and irrespective of their age, while Marcus (1942) cultured the inner sound tissue of seeds and fruit of a number of different plants and obtained fungi, yeasts or bacteria from some kinds but found other tissues sterile.

Results of studies on a new potato disease led Sanford (1948) to an investigation of bacteria which he found to inhabit not only the stems, stolons and roots of the affected plants but those of normal healthy plants also. A large number of isolations were made from the general vascular regions of the stems of healthy potato plants of various ages, as well as from the stems of *Phaseolus vulgaris* and the tap roots of *Medicago* spp. and *Melilotus* spp. Sanford was able to show that several kinds of bacteria may commonly exist in such regions of the plant. Although the majority of the isolates were not identified, one of them, frequently present in the interior of older potato stems, proved to be *Agrobacterium radiobacter*. This same bacterium, although incapable of any nitrogen-fixing activities is reported as commonly associated with *Rhizobium* in the leguminous root-nodule.

Since 1948 relevant literature has appeared supporting these claims of the presence of a microbial population existing within healthy plant tissue. That the interior of normal potato tubers and storage organs are not sterile has been ably demonstrated by Tervet and Hollis (1948) and by Hollis (1951) who observed the presence of a mixed bacterial flora including clostridial (anaerobic) types. A remarkably constant type appearing in these isolates was identified as *Bacillus megatherium*, an aerobic spore-forming bacterium and one of the most common of is type occurring in the soil.

Although perhaps beyond the scope of this survey it is of interest to note that micro-organisms other than bacteria have been found to be present within apparently healthy plant tissue. From America, Hyde (1950) has reported subepidermal fungal hyphae to normally occur in grain from nearly all wheat-growing areas of the world. The amount of mycelium present is reported to differ widely and there are indications that the degree of infection is dependent on atmospheric humidity during ripening of the grain. Whatever its cause, the presence of this mycelium has been found responsible for the heating of stored cereal grains and attendant detrimental effects.

Finally Thomas and Graham (1952) have reported bacteria in the stems of apparently healthy pinto beans. The striking fact revealed by this investigation is the passive existence in the bean plant of bacterial types otherwise pathogenic to it. When the pathogenicity of all the isolated types was tested typical symptoms of bacterial blight and wilt developed under glasshouse conditions.

And from my own studies, I was able to show the existence of bacteria in healthy clover roots. Of the bacteria types successfully isolated, five types were subjected to further study to determine their morphological, cultural and biochemical characteristics. The identity of three of these bacteria was tentatively established. One closely resembles *Aerobacter cloacae* which was recorded by Hollis (1951) as occurring in healthy potato and legume tissue. Another was similar to *Bacillus megatherium* which the same worker found to be a common type in the stems and roots of potato plants, while another resembling *Flavobacterium rhenanus*, has not yet been recorded from healthy plant tissue, as far as is known. The remaining two bacterial types studied have not yet been identified. The existence of these bacteria supplies additional evidence in support of the comparatively recent finding that normal halthy plant tissue is not sterile but supports a mixed bacterial population of passive habit.

Obituary

MISS E. PARTRIDGE

The death occurred in Christchurch on Wednesday, after an illness of more than three years, of Miss Elsie Millicent Partridge, daughter of Mrs. A. Partridge, Weston Road. Miss Partridge, who will be remembered among tennis players of an earlier day as a fine representative player, was educated at the Christchurch Girls' High School and studied later at Canterbury University College, gaining there her B.Sc.

She was awarded her university blue in 1923. It was during this period that she represented the college and Canterbury. She was subsequently a representative player for Otago.

Teaching Post

Miss Partridge was on the staff of Amberley House for a few months before going in 1928 to the post of first assistant at Archerfield Girls' School, in Dunedin. She was deputy principal during the absence of Miss Black abroad.

Resigning in 1937, she went to Britain on a visit. War intervening, she undertook the driving of an ambulance, and later business work to relieve a man for the forces.

Studied Bacteriology

Miss Partridge subsequently spent four years in the medical school at the Charing Cross Hospital, as a student of bacteriology and, after her return to New Zealand in 1947, was for three years at the Wanganui Hospital bacteriological department, during which time she gained the New Zealand diploma in her subject. Her illness interrupted her chosen profession shortly afterwards.

Keenly interested in outdoor recreation such as ski-ing and tramping, Miss Partridge was also an original member of the Harmonic Society, and had studied singing and the violin.

IMMUNITY IN VIRUS DISEASE

L. B. Fastier

Virus Research Laboratory, Otago Medical School.

The present inability of antibiotics to favourably influence the course of nearly all viral infections emphasizes the importance of both natural and artificial immunity in the latter diseases. Containing nucleoproteins within a formed structure, viruses act like any antigen and stimulate the formation of antibodies when inoculated living or dead into a mammalian host. These antibodies, while differing in no way from their bacterial counterparts must, to be effective, act within an environment the limited intracellular nature of which poses almost insuperable problems from the therapeutic aspect. For instance, it has been shown that once a mouse has been infected with the virus of Western equine encephalomyelitis, then the inoculation of specific antiserum even in amounts equivalent to half the total blood volume failed to influence the lethal outcome. While recent reports of Hammon et al on the successful use of gamma globulin in poliomyelitis are of great interest, it cannot be too strongly emphasized that these trials indicate prophylactic value only. Furthermore, until more is known of the epidemiological occurrence of the three serological types of poliomyelitis virus, most authorities recommend a conservative approach towards mass use of this expensive blood product.

The majority of virus infections, with the notable exceptions of influenza, the common cold and perhaps dengue, confer a long-lasting degree of immunity after natural or experimental exposure. Thus second attacks of poliomyelitis, variola, mumps, etc., are a comparative rarity. These viruses were chosen to illustrate not only the variable degrees in an immune response but also to attempt to clarify a number of epidemiological observations which hitherto had defied explanation by conventional concepts of immunity. Firstly, reference to the infectious process in the above disease immediately suggests that those confined to the upper respiratory tract, by virtue of their narrow tissue tropism and absence of blood stream invasion (viraemia), do not possess the intimate contact with the reticuloendothelial system exhibited by infections with longer immunity rates. Secondly, we have the vast problem of multiplicity of antigenic types. When a virus is antigenically homogeneous, such as rabies, yellow fever or vaccinia, then provided an attenuated strain can be prepared by laboratory manipulation, its use in a human population may confidently be expected to provide an effective immunity against all strains of the one virus. It is perhaps significant that of all human virus diseases only these three have, as yet, been successfully combatted by prophylactic vaccination. On the debit side of the picture are certain conditions, notably influenza, where the antigenic structure and instability of virus types place any vaccination procedure almost on an empirical basis only. Analysis of laboratory-isolated strains which have accumulated since 1934 has suggested a definite chronological variation in the serological make-up within one broad antigenic type.

While partially effective immunity may result from vaccination with a strain recently isolated, the possibility always exists of an epidemic due to an entirely new viral sub-type for which these antibodies would be quite ineffectual. A potential catastrophe of this nature might have occurred in 1947 when prototypes of the now current A prime strains were isolated from severe influenza epidemics in vaccinated populations.

The problem of virus immunity is still further complicated by a process aptly described as 'the masking phenomenon.' Found both naturally and experimentally in herpes febrilis and vaccinial infection (and in all probability much more widespread), a state can exist whereby active viruses may be isolated from a solidly immune animal. In many humans the former infection is characterized by skin lesions recurring in the same anatomical position in response to some non-specific physiological disturbance such as menstruation or the febrile attacks accompanying either typhoid vaccination or the therapeutic use of malarial parasites. These conditions have been reproduced in adult rabbits, where a fatal encephalitis was precipitated by an anaphylactic shock nine months after infection with herbes febrilis virus. At autopsy the virus was recovered from the central nervous system. The obvious significance of these findings is that the balance between herpes antibody and the infectious agent (the latter obviously persisting throughout the whole period) is so finely adjusted that dissociation may be accomplished by the ordinary physiological or pathological processes to which humans or lower animals may be subjected during their lifetime. A further example of a masked infectious state is shown by the dissociation by physical means of vaccinia virus from homologous serum antibody one year after infection of rabbits. However, it is unnecessary to quote these apparently academic experiments when, to a person engaged in diagnostic work, each attempted virus isolation is complicated by this phenomenon. The early production of effective antibody levels, together with high non-specific inhibitory titres in both serum and the secretions and excretions of human subjects are complications which illustrate the present difficulties and inadequacies of our virus isolation techniques.

To summarize the above material before proceeding further, we may therefore consider that antibodies produced in response to contact with virus protein differ neither chemically nor physically from those produced by bacteria. In every instance the strict specificity so characteristic of antibodies is preserved. The lengthy duration of immunity resulting from many virus diseases may possibly be due to persistence of the virus throughout the whole period. While such a condition obviously does occur under certain conditions, it would seem more probable that the original agent, following modification by the first immune response of the host, to a non-infectious state, is maintained in this latter condition. Needless to say his process would not impair the prosthetic groups of the viral protein which act as templates for the continued synthesis of antibodies.

Viral antibodies, like their bacterial counterparts, not only confer a considerable degree of immunity upon an individual but also provide

the most satisfactory method of studying the epidemiology of a specific disease. Where a virus can readily be communicated to common laboratory animals or to fertile hen eggs, the resulting infected tissue suspensions constitute very effective antigens in the complement fixation test. By ultra-violet irradiation such antigens may be rendered non-infectious, and can thus be utilized with no special precautions in a routine bacteriological laboratory. The neutralization test as its name implies depends, however, upon the ability of serum antibodies when mixed with virus and subsequently inoculated into susceptible animals, to specifically inhibit the infectious process. This procedure is obviously beyond facilities available in a normal pathology department and while still useful in certain poliomyelitis studies, is now regarded as being of confirmatory value only.

It is apparent that certain diseases merit specific techniques. Either the virus cannot readily be transmitted to common laboratory animals, as in poliomyelitis and serum hepatitis, or diagnostic procedures which depend upon some special function of the virus particle may find application. In the former instance *in vitro* titrations of certain cytopathogenic poliomyelitis strains have met with some measure of success. These methods depend upon the property of such viruses to induce degenerative changes in fibroblasts proliferating from small fragments of monkey testicle embedded in plasma clots. Type specific antiserum prevents such changes while heterologous serum has no inhibitory effect and necrosis proceeds. Methods which depend upon some specific property of the virus particle are almost entirely confined to the Influenza-Newcastle disease-Mumps group. Here, the attachment of the virus particle to so-called receptor areas on the surface of human or avian erythrocytes results in visible haemagglutination. Pre-mixing of virus with antibody (under controlled conditions) prevents the agglutination of subsequently added red cells. Thus the highest serum dilution to prevent red cell clumping in the presence of excess virus represents the haemagglutination-inhibition titre and, as such figures are proportional to the degree of immunity developed by an individual for each specific virus employed in the titration, the method offers a simple procedure for the retrospective diagnosis of influenza. It would not be an overstatement to say that this one technique has contributed as much towards an understanding of the epidemiology of influenza as the perfection of chick embryo culture methods have advanced our knowledge of virus diseases in general.

The foregoing comments illustrate a few of the problems which attend a study of immunity in virus diseases. At the moment an efficient immune mechanism is still the best saviour of the human race from future plagues of these maladies. Furthermore, as our concepts of such diseases broaden, there is every likelihood that their eventual control may be accomplished by judicious use of these facts. This may be illustrated by the results of a study on the incidence of paralytic and non-paralytic poliomyelitis cases in populations of different ages and economic groups. It was found that serum neutralizing antibodies to

the Lansing strain (Type 2 poliomyelitis) became apparent at a much earlier age in families of a low economic class. That these antibodies conferred considerable immunity upon this whole group was shown by the lower paralytic rate in later life. The suggestion has been advanced that although the incidence of non-paralytic intestinal infections may not have been reduced, the antibody level was sufficient to prevent a 'break through' with the resulting virus involvement of the central nervous system. In the higher economic group (younger children presumably attending more select boarding schools) the rise in protective antibody occurred relatively late (if at all) in adolescence, and significantly, it was this group that experienced the highest attack rate of paralysis. This appears to be directly related to the absence in early childhood of an inapparent infection which, fostered by conditions of overcrowding and poor hygiene, was to subsequently protect the lower class group from infection. Burnet has summed up the situation by considering that the most disastrous state which could befall a population would be the development of a community of totally susceptible individuals. It seems ironical to think of modern hygienic conditions as fostering both the spread and pathogenesis of poliomyelitis, yet it is not overemphasizing the position to say that each improvement in the standard of living simultaneously increases the chance of higher paralysis and fatality rates should the younger members of such communities be exposed to infection later in life. It is obvious that this one feature of poliomyelitis, amongst the many which could be quoted for each virus infection, provides an admirable example not only of the impact which changed concepts may have on the epidemiology of an infectious process, but also in the manner in which potentially effective vaccines might be utilized to their best advantage.

NEWS AND NOTES

Wanganui:

Mr. Alan Harper was married a few months ago to Sister Cassidy—both ex-Invercargill.

Miss Enid Hicks was married recently to Dr. Alan Austin—now of Napier Hospital.

Miss M. Burtt has returned from a trip abroad during which time she spent several months with the North London Blood Transfusion Centre.

Miss Joan Wilkinson is due back from England in November. She had several months' experience in the Chelsea Woman's Hospital Laboratory.

Auckland:

Mr. D. Whillans, Principal Hospital Bacteriologist, recently completed 25 years' service in Laboratory work. At a special morning tea to mark the occasion Mr. Whillans referred to the amazing advances which had taken place in Laboratory work in the last 25 years.

Mr. John Sloan, Hospital Bacteriologist, Cornwall, became the amazed father of twins in May!

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES.

Theoretical Paper.

June 12th, 1953. **9.30 a.m.—12.30 p.m.**

1. Discuss the factors influencing the growth of bacteria on artificial culture media.

2. Describe in detail one method of estimating chlorides in a specimen of cerebrospinal fluid.

3. Describe briefly your method for each of the following tests:—
   (a) Platelet count.
   (b) Erythrocyte sedimentation rate.
   (c) Bleeding time.
   (d) Icterus index.

4. Give a concise account of the various procedures you would undertake in the examination of a specimen of pleural fluid.

5. Describe your technique for A.B.O. blood grouping. List the apparatus and materials required. What are the sources of error? How can they be avoided?

6. Describe details in the preparation of a litre of nutrient agar from raw materials.

PRACTICAL EXAMINATION

2 p.m.—5 p.m.

Section A (1½ hours)

1. (a) Report on the organism on the culture "A."
   (*C. diphtheriae*).
   (b) Identify the Gram negative non-lactose fermenting bacillus, in pure culture, from a specimen of faeces. It is on an agar slope "X."
   (*This examination will be concluded on Saturday morning*).
   (*Shigella sonnei*).

2. (a) Examine the urine for albumin and sugar (qualitative). Give details of technique.
   (*Albumin and trace of sugar present*).
   (b) What is the 24 hours excretion of glucose if 4.7 ml. of a 795 ml. 24 hours specimen reduces 25 ml. of quantitative Benedict's Solution.
   Show all calculations.
   (8.45 *gms.*)
   (c) Report on the deposit of the urine.
   (*R.B.C.'s. Leucocytes, Hyaline and Granular Casts*).

Section B (1½ hours)

3. (a) Estimate the total non-protein nitrogen on the sample of blood provided.
   Use the Standard provided—then.
   $\frac{\text{Reading of Standard}}{\text{Reading of Unknown.}} \times 30$ equals N.P.N. per 100 ml. of blood.
   (b) You are given a N/10 solution of HCl. Find the normality

of the solution of sodium hydroxide provided.
Detail all stages of your work and give calculations.
(*NaOH equals* 40 *HCl equals* 36.5)

4. Stain the two blood films provided. Describe your technique for staining.
Write a report on both films.

5. To be done on Saturday morning.
(*Five culture plates for identification of organisms and five stained microscopic slides for identification.*)

ORALS.

Some of the questions asked were as follows:—General principles of autoclaving, disposal of cultures, disposal of sputum, Bordet-Gengou medium, Dorset's egg medium, Lowenstein's medium, treatment for acid splashed into the eye, Gregerson's test for occult blood, Loffler's Methylene Blue stain, Neisser's stain, meaning of pH.

DEPARTMENT OF HEALTH.

Certificates of Proficiency in Hospital Laboratory Practice. Wellington Hospital, September 3rd, 1953.

EXAMINERS

Dr. T. H. Pullar, Dr. J. O. Mercer.

1. What is the range of error inherent in red cell counts? What methods other than by the ordinary red cell diluting pipette are available to enumerate erythrocytes. Describe one of these alternative methods and state its advantages and disadvantages.
2. Describe the laboratory procedures which may be used in the diagnosis of amoebic dysentry. What parasitic ova may be found in faeces while searching for amoebic cysts?
3. Write brief notes on
   a. The detection of occult blood in the faeces.
   b. The diagnosis of lead poisoning by laboratory methods.
   c. The assessment of the degree of jaundice.
4. What do you understand by the term serum proteins?
What methods are available for their estimation?
Outline a method with which you are familiar.

PRACTICAL EXAMINATION.

Time, 3 hours.

1. Examine and report on cultures 1 to 4.
(*Cl. welchii, B. subtilis, pneumococcus, C. diptheriae.*)
Identify plates 5 and 6 by cultural and serological examination.
(*S. sonnei S. flexner.*)
2. (a) Stain and report on slide X which is from the centrifuged deposit of a specimen of cerebro-spinal fluid.
(*Meningococcus.*)
   (b) Report on the deposit of the urine Y.
(*Granular casts*).
   (c) Make a differential count and report on the three stained slides of peripheral blood provided.

(*Myeloid leukaemia, Hypochromic anaemia, Infectious* Mononucleosis).

(d) *Examine and report on the moist blood film for parasites.* (*Microfilaria*).

3. Perform a post-mortem on the guinea pig provided. Report on your findings. This animal was inoculated with a concentrated specimen of gastric washings six weeks ago.

ORALS.

The following are some of the questions asked.

Dr. Mercer: Coombes Tests and erythroblastosis; intravenous solutions; stills; pyrogens; Hb. estimations; Wasserman Reaction; Rideal-Walker and disinfectants; autogenous vaccines; Tb. concentrations; Lowenstein's medium; Paul Bunnell.

Dr.Pullar:Calcium levels in serum; urea estimations; acid and alkaline phosphatase; yellow fever vaccine; diphtheria toxoid; Tb. concentrations; spectro-photometer; Coombes tests; CO2 combining power; potassium estimations; cholesterol.

Successful candidates in this examination were:—

GRAY, L. J.—Southland Hospital.
MAIN, B. W.—Christchurch Hospital.
RUDD, B. P.—Auckland Hospital.
RYAN, K. M.—Palmerston North Hospital.
SEWELL, D. E.—Timaru Hospital.

MINUTES OF THE NINTH ANNUAL CONFERENCE OF HELD AT CHRISTCHURCH ON 16th & 17th JULY, 1953. THE N.Z. ASSOCIATION OF BACTERIOLOGISTS (INC.),

The President, Mr. D. Whillans, introduced Mr. V. C. Lawn, Chairman of the North Canterbury Hospital Board, who was present to extend a welcome to delegates.

The President said that Mr. Lawn was also a vice-president of the N.Z. Hospital Boards' Association, and a member of the Executive of that body, and had a reputation for close co-operation with the Staff of the North Canterbury Hospital Board institutions.

The President noted that Mr. Lawn had received the Coronation Medal in recognition of his services to the community, and then called on Mr. Lawn to speak to delegates.

Mr. Lawn:

Mr. President, Ladies and Gentlemen:

I feel honoured that I have been invited to say a few words to you on this your 9th Conference. There is a saying, "Cut out the cackle and get on with the job," and that is precisely what I intend to do, so just let me say that on behalf of my Board I extend a very hearty welcome to you.

I understand that it is now six years since you favoured us with a visit, and I trust that your Conference will be both profitable and pleasurable. Glancing through your programme, I see that you will have many addresses, some demonstrations and some time for pleasure. I am sure you will enjoy your visit to Canterbury, although you have come to us in mid-winter. If there are any representatives from the Waikato area, we would like to express our sympathy through you to all those folk who have suffered

so much in the recent floods. There must have been a great deal of hardship, and we know there has been much damage to buildings and land through the unfortunate circumstances. We from the City of the Plains trust that an early restoration to normal conditions will soon prevail. Please take back our best wishes and kindly thoughts.

I presume that most of you come from public hospitals, and a few from private laboratories, and there are perhaps some representatives from colleges. There is no need for me to stress the importance of your work which calls for much exacting and painstaking concentration in the diagnosis of hospital and other cases. Yours is not a spectacular occupation, as you work "behind the scenes," but in these days of so much specialisation, there is an ever increasing demand on your services.

Now you have gathered here to confer and pool your knowledge, and perhaps for some of you it is your first experience of a conference. May I suggest to such "Don't be afraid to get up and voice your opinion," and thus give others the benefit of your knowledge. I have attended many conferences and I have occasionally heard the remark, "Well I didn't get much out of *that* conference," and there may be delegates here who have had similar experiences. Let me say this, that unless you have come here prepared to put something into this "get together," you cannot unselfishly expect to get anything out of it.

Now, as most of you come from public hospitals, I wonder if I may be pardoned for speaking as a vice-president of the Hospital Boards' Association and representing the Industrial Union of Employers, in voicing the general opinion of all Boards that we are very concerned at the ever-increasing cost of hospitalisation of the sick. I have attended meetings on costs investigation, and I have also sat in Conciliation on Industrial awards, when there are claims for increased wages and improved conditions, with more and more demands for penal rates under certain circumstances. While I stand full square for adequate payment for services rendered, I do feel that the time has surely arrived when there should be some stability in this direction. You are probably aware that from 60 to 65 per cent. of hospital costs are in salaries and wages, and Boards are tied in this direction, but the point I wish to make is that costs must be kept at a minimum otherwise our economic structure must deteriorate. You, as employees, will probably say, "Well, what can I do about it, I work my full 40 hours, or whatever my span of duties may be, and I do my best," but does it ever occur to you that you can reduce costs by exercising care in the use of equipment, much of it very expensive, and by economy in the use of power, stores, etc. I could quote cases of careless handling of equipment and services and wastage in materials, etc.

Another avenue for reducing costs is by stepping up the output, and this can be achieved in many ways which must be obvious to you. In these days of a short working week with high wages, concentration on the job and strict compliance with the scheduled hours of duty and spell breaks, is essential.

May I appeal to all executive officers to see what you can do in the desired direction, and to all those in what might be termed the "rank and file" positions, I say, "Be loyal to your employer and let 'pride of service' be your watchword." Perhaps in your general discussion at this conference, or more particularly when you get together on the odd occasions, you may keep these thoughts in mind.

Now I am afraid I have touched on something which you may regard as beyond the scope of this gathering, but it is an aspect which could well be one for consideration at the proper time.

May I take this opportunity to tender the greetings and best wishes from the Hospital Boards' Association and from the members of our board.

At a recent conference of Hospital Boards, a remit from Wanganui that staff conferences be held biennially, although well received, was

eventually lost. I feel that I am quite safe in saying that Boards generally and the Health Department, welcome these conferences because it is appreciated that the pooling of knowledge in various ways tends to raise the standard of efficiency and broaden the views of the participants, but we must all realize that here also the cost of such gatherings is continually on the up-grade. I therefore seriously suggest to you that if *annual conferences* are to continue, some tangible result must accrue, otherwise it might be claimed that the actual cost and loss of time by those taking part may not be fully justified. I do however appreciate that the full value and subsequent result of conferences is somewhat difficult to assess.

In repeating my welcome to you may I express the wish that this Conference may be both successful and pleasurable and that at its conclusion you will feel that your journey to the Garden City has been well worth while.

The President thanked Mr. Lawn for his welcome to delegates, and assured him that his remarks would be heeded by all Laboratory workers. He then called on Dr. D. T. Stewart, Director of Pathology, Christchurch Hospital, to open the Conference, and address delegates.

Dr. D. T. Stewart:

Inaccuracy and Integrity in the Laboratory.

The excuse for a Conference such as this is the comparing of laboratory methods and the reporting and evaluation of the results of such methods.

In his introduction to a number of the British Medical Bulletin entitled "Measurement in Medicine," Sir Henry Dale used these words: "The change . . .from qualitative impression to quantitative measurements so effective in promoting the conversion of clinical medicine from an empirical art into an experimental science seems to have come from two main directions. The first of these, in general terms, is the adaptation to clinical uses of physical and chemical methods of measurement."

But for this application of science to medicine you and I would not be here today and medicine would still be in the dark ages. This should give you all a warm sense of self satisfaction, but before you settle deeper in your chairs let us consider Sir Henry's second point. This was: "The growing recognition of the need to control the significance of a large part of medical evidence by statistical methods. This, again has strengthened the sense of common action, with a common aim, between the laboratories and the clinics, through their common experience of the need for this bracing discipline, with its corrective influence on practices and assumptions to which both had been addicted."

Let me hasten to say that I am no statistician—indeed I have always been bad at arithmetic: but I ask you to consider with me the sources of inaccuracy and the importance of integrity in the laboratory, lest we be accused of subscribing to what I have heard described as almost a N.Z. motto, "Near enough is good enough."

The material we deal with is biological and being human we must therefore be content with miserably small samples—like Shylock we cannot cut off a pound of flesh for analysis. One human being differs from another and our sample may be affected by many factors—the age and sex of the patient, exercise, a recent meal, how long the vein was compressed and the sex of the technician. Thus our material and therefore our results are subject to a fundamental *biological and sampling variation.*

But this is no excuse for saying that any old method is good enough for clinical purposes: "On the contrary (to quote the Editor of the Lancet) the very extent of variation from case to case makes its essential that the laboratory shall not add a large chemical scatter."

When we come to consider laboratory methods we must first define accuracy, precision and reliability.

Accuracy: Is the comparison of the observed and true value.

Precision: Is the degree of variation in results by a method when the

same sample is run repeatedly—i.e., the reproducibility of what is observed.

Reliability: Is the ability to maintain accuracy and precision into the future: Checks over a period are required to establish this.

As regards *accuracy* the biological variation I have mentioned means that often there *is no* true value with which to compare our observed value. It is necessary however for us to choose methods which are the best, just as we must choose suitable equipment—for example appropriate pipettes.

A certain laboratory test may have poor accuracy (e.g., the Folin-Wu method for blood sugar estimates reducing substances other than glucose) but it may have good *precision*. It is important that we know the degree of precision of any tests we use.

The precision or "confidence limits" as some call it of a test can be determined by doing say 30 tests on a single specimen or duplicates of tests on 30 specimens, thus finding the range of variation of results.

The establishment of this range of variation for any test is important first to the laboratory. It is of no use setting out to use a test the inherent range of variation of which is greater than the range of clinical or biological variation. A variation of 10% would be all right for blood sugar, but more than 3% would not do for serum sodium. Therefore the precision of a test is of prime importance in considering whether it should be adapted for use in the laboratory. For example it has recently been amply demonstrated that the errors inherent in doing a red cell count are so great as to make this test unsuitable for routine use. A special technique will lessen the error, but note that the error of the routine method is inherent—the best observer cannot lessen the error, but a poor one can add his own personal error. The red cell count is best replaced by the newer methods of haemoglobinometry and the packed cell volume both of which have a greater precision. Secondly, precision is of great importance in our relations with the clinicians. It is important that they should be informed as to the variation inherent in any tests so that they can assess the significance of day to day changes. For example it has been shown that if a Hb. reading is found to be 50%, the precision of a certain method allows only that the true value lies somewhere between 44 and 56% and that a change of less than some 9% in isolated readings is not significant. We cannot too often impress this point on our clinical colleagues: there is much to be said for indicating the precision of a test whenever we report a result.

Now how can we control the precision of a test, that is ensure that it has RELIABILITY? This can be done usually by the employment of controls and standards—I feel too seldom used. It is possible now to preserve by deep freezing large volumes of blood or serum to be used in small amounts for control of laboratory estimations of many of its constituents and it should be possible for base laboratories in this country to imitate what the M.R.C. in England are doing with haemoglobin, that is to make available to smaller and isolated laboratories standard samples of blood and serum and of solutions for control of precision.

Both in England and the U.S.A. the use of *control charts* has been recently advocated. This involves a chart for each method with a central line representing the value of the standard and on either side lines indicating the limits of variation for the method or the ranges above and below which not more than 10% and 1% of results should fall. The results of estimating the standard with each batch of tests is recorded on the chart, which should show a scatter within the ranges or limits of variation. Persistence of results on one side of the true value indicates a developing fault. The test fails in precision when the figure for the standard falls outside the limits of variation. The source of the trouble must then be investigated. Such control charts might well be more widely used. I now come to Personal inaccuracy and to the second part of my title, *Integrity* in the Laboratory.

I hope I have indicated above that in our work we are up against both biological variation and inherent lack of precision in many of our methods. These two inaccuracies must not be magnified by personal inaccuracy. Lack of proper supervision and training, inadequate facilities and staff may all make for poor work, but we must be constantly on guard lest our personal accuracy and honesty lapse. We must remember that the health, even the life, of the patient may depend on the result of the observation we are making. The test must be repeated or a new specimen obtained if there is any doubt as to the proper performance of the test.

In my personal work, largely the examination of histological sections, it is often tempting to write a report on incomplete sections rather than go to the trouble of cutting more pieces or asking the technicians to cut deeper sections which may or may not reveal something or demonstrate that a cancer has in fact been incompletely excised. This is when I try to pause and think—am I doing the best for the patient?

I would like to hold up to you an ideal of integrity: The late Dr. A. B. Pearson, former Director of Pathology in this Hospital. He was thorough and painstaking in his work and honest and sincere in his opinions.

Think every time before you write down your observations—have you been honest to yourself and done the best for the patient and out of the corner of your eye see a motto hanging on the wall *"Near enough is not good enough."*

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The President thanked Dr. Stewart for his most interesting and instructive address, and hoped that all laboratory workers would profit from it.

The President's Address.

Now that we have reached our Ninth Conference, we have come to the stage where there has been much accomplished—indeed it would have been a brave man who could have looked forward and prophesised what has, in fact, come to pass in Hospital Laboratory Practice in New Zealand. This being so, it is a necessary corollary that the gains yet to be made must seem small in comparison with the past. They will, in fact, be in the nature of a polishing up, or slight re-arrangement of those things accomplished and no striking advances can probably be expected.

The members of the Council who have had the honour of representing you and serving you in these years have been a relatively small band and one upon which the hard work of making the Association a success has fallen. It is my belief that a living Association is one in which the members of the Executive give their service and are then gradually replaced, member by member. This ensures that the older members pass on to the younger the tradition of the Association so that not only has the Association the constant infusion of new talent but also the experience of the past to weld into a dynamic future.

It is at this stage that there lies the danger of the rise of factions in an Association, but I am happy to say that it has been, and I hope ever will be, one of the strengths of this one, that differences which must inevitably arise in a growing organisation have been talked out to an amicable conclusion.

It is here, too, that a duty devolves on all members of the Association —that is to perform the work with scientific detachment, keen interest and

the technical dexterity that is the mark of a good worker, but not only that, to remember that if we stand still and learn nothing new, we fall back in the world of Science, and are sadly outdated in even a short time.

Ladies and Gentlemen, I will not take up your time further, though this subject is close to my heart, but will ask you to consider these matters further when the Conference is over and you are back in your own Laboratories.

Roll Call:

The following delatges attended the 1953 Conference. Messrs Whillans, Auckland; Olive, Wellington; McKinley, Waipukurau; Murray, Christchurch; Buxton (snr.), Wanganui; Buxton (jnr.), Oamaru; Burtt, Greymouth; Austin, Dunedin; Dr. Fastier, Dunedin; Messrs Bloore, Blenheim; Ekdahl, Gisborne; Rose, Christchurch; Hawke, Nelson; Harper, Wanganui; George, Rotorua; Ellison, Wellington; Jarratt, Palmerston North; Hilder, Christchurch; Ronald, Whangarei; Scott, Thames; Foster, Christchurch; Reynolds, Wellington; Josland, Wallaceville; Misses Whelan, Wellington; M. Smith, Wellington; White, Ashburton; Gray, Invercargill; Mrs. Robertson, Christchurch; Messrs Thompson, Invercargill; Samuel, Dunedin; Morgan, Dunedin, Misses Crozier Lincoln College; Scott, Auckland; Byres, Auckland; Davies, Hamilton; Mills, Rotorua; Kennedy, Hamilton; Mr. Mann, Hamilton; Miss Jury, Tauranga; Miss Corsbie, Tauranga; Messrs Walsh, Auckland; Bilkie, Auckland; Patterson, Auckland; Connolly, Auckland; Kershaw, Balclutha; Jenner, Hawera; Mead, New Plymouth; Gibson, Christchurch; Adamson, Christchurch; Till, Christchurch; Miss McRae, Christchurch; Messrs Cannon, Christchurch; Hutchings, Palmerston North; Main, Christchurch; Diggle, Westport; Miss Curtiss, Christchurch. Several other members of the Christchurch Laboratory Staff attended as their duties permitted.

Apologies:

Mr. Rush-Munro, Mr. Curtis, Miss Taylor, Mr. Saunders, Miss Wylie, Mr. Masters, Mr. Carruthers, Sister Paula, Mr. Rankin (for H.B. Hospital Board Staff); Miss Grey, Mr. Pierard, Mr. Reid, Mr. Kennedy, Mr. Peddie.

Deceased Members:

Dr. A. B. Pearson, Honorary Member (Christchurch); Miss Partridge (Christchurch, formerly of Wanganui); Miss Penrose (Medical School, Dunedin).

Delegates stood in silence, as a mark of respect.

The Hon. Secretary was instructed to convey the sympathy of the Association to the relatives of the deceased members.

—(Olive-Buxton).

Minutes of Conference—1952.

Published in the Journal, October 1952, and taken as read. The minutes were confirmed. —(Buxton-Murray).

Business Arising From the Minutes:

Recognition of the Final examination by the Public Service Commission.

Mr. Olive explained that he had discussed this matter with the appropriate authorities but there had been no tangible results so far.

RESOLVED: "That the executive pursue this matter with the Public Service Commission." (Josland-Till).

THE ANNUAL REPORT was presented by the Hon. Secretary and was received. —(McKinley-Ellison).

THE BALANCE SHEET was presented by the Hon. Treasurer and was adopted. —(Olive-Cannon).

Election of Officers, 1953-4.

President: Mr. Whillans.
Vice-Presidents: Messrs. Adamson and Buxton.
Hon. Secretary: Mr. McKinley.

Hon. Treasurer: Mr. Patterson.

Members of Council: Messrs. Reynolds, Rush-Munro, Samuel, and Miss Scott.

Moved—"That the ballot papers be destroyed."
—(Ellison-Adamson).

The President regretted Mr. Olive's departure from the Council and thanked him for the work he had done for the Association.

Mr. Ellison said he was very sorry to see Mr. Olive's services lost to the Association, and recalled how Mr. Olive had carried most of the burden of representing the Association in the Industrial Union dispute, and he moved—"That this Conference place on record the services of Mr. Olive to the Associations." —(Ellison-Whillans).

Carried with acclamation.

Mr. Olive expressed his appreciation of the kind remarks made, and said he was always pleased to do all he could for the Association. He wished the new Treasurer well in his office.

Mr. Ellison considered that it would be an advantage for voters to have some information regarding candidates for office and he recommended this thought to the incoming Council.

The President announced that this was last year of office, as he considered others should move up in office. He considered it could be difficult to give "thumb-nail" sketches of candidates. He warned members of the dangers of "block voting" by laboratories, with consequent retaliation. The Association comes first, and must continue to do so.

Mr. Till endorsed Mr. Ellison's remarks and it was moved. "That a short biographical sketch of candidates for office be supplied with voting papers." —(Till-Ellison).

The Hon. Secretary was instructed to write to Messrs. Saunders, Rush-Munro, and Horner expressing the wish for their speedy recovery.
—(Murray-Till).

The President:

Re Graded Officers.

In Auckland grading is known, as the information is not kept confidential by those persons who are graded. The Grading Commitee is not able to supply a list of graded officers.

Mr. Ellison briefly explained the working of the Grading Committee and said that a Graded Officers' Salary could not be disclosed by that Committee, but the individual concerned could disclose his salary if he felt so inclined.

Mr. Bloore: If wishing to apply for a different position, how does the applicant know the grade of the position?

Mr. Ellison: Apply to the Director-General of Health, and the Hospital Board concerned.

Mr. Olive: In the case of a *new* position, the Director-General could probably give some indication of the likely grade, after considering the applicants' experience and qualifications.

Mr. Buxton: Would like to see grades published.

Mr. Whillans: Outlined the functions of S.A.C. as distinct from the knowing grades—but realised that comparisons can be misleading. He explained that a grading is permanent unless circumstances change.

Mr. Ellison: Grades are reviewed yearly, which is sufficient.

Mr. Olive: Review of one, means review of all.

Mr. Whillans: Otulined the functions of S.A.C. as distinct from the General Committee and Grading Committee.

Mr. Olive: Moved—"That the Director-General of Health be asked that any proposed alteration to Hospital Employment Regulations (Laboratory Workers) be referred to the N.Z. Association of Bacteriologists for their information and comment if necessary." —(Olive-Meads.).

Mr. Whillans: In answer to Mr. Adamson, stated that the basic wage is paid by law to those over the age of 21 years.

Mr. Adamson: Some people over the age of 21 years are not being paid the Basic Wage.

Mr. Ellison: Moved—"That the Association support individuals as required in approaching the Board concerned re payment of the Basic Wage." —(Ellison-Buxton).

Mr. Whillans: Considered individuals should take the necessary action to protect themselves.

Mr. Rynolds: Asked if the Association were satisfied with the name Bacteriologists, and would not "Technicians" be a better name.

Mr. Ellison and Mr. Whillans traced the past history of the name of the Association, and did not think any proposed change would be welcome.

Mr. Whillans asked if any change was desired, and on the voices it was obvious that members preferred the existing designation.

Mr. Mead: Some trainees are not members of the Association. Were they not encouraged to join?

Mr. Whillans: The Association does not campaign for members. Members could explain the advantage of membership to new trainees.

THE ESSAY PRIZE—1953: This was won by Miss J. Grey, New Plymouth. The President congratulated Miss Grey on her second success in this field.

Conference, 1954, Wanganui.

—(Buxton-Whillans).

HONORARIA:

Hon. Secretary	£5	5	0
Hon. Treasurer	£3	3	0
The Editor	£3	3	0
The Auditor	£3	3	0

—(Adamson-Bloore).

EXAMINATIONS: It was not considered practicable to hold examinations only once a year, as in the case of the Intermediate Examination this could mean 20 candidates, and only Dunedin could cope with this number.

Mr. Ellison recommended to Council that representatives to meet the Pathological Society be appointed from members near to the venue of the meeting.

SALARIES ADVISORY COMMITTEE: There were no further submissions from Conference.

The Association's position in the dispute with an Industrial Union. There were no further moves in the last year.

The Meeting with the Executive of the Pathological Society.

The President and Hon. Secretary represented the Association. (21st May, 1953).

Following this Meeting the Director-General of Health informed the Association:—

(1) That the Pathological Society does not favour the appointment of a Senior Bacteriologist to the panel of examiners for the final examination:

(2) The Department of Health will assist in any way possible in the Association's move to reach agreement with the I.M.L.T. England, re reciprocal arrangements for qualifications.

(3) The question of the proposed Higher Examination be left to the Association.

The present content and organisation of the final examination is to be reviewed by the incoming Executive of the Pathological Society.

(4) The Department of Health will set up a panel to decide the date, venue and examiners for the Intermediate and final examinations. One representative each from the Department of Health, The Pathological Society, and the N.Z. Association of Bacteriologists.

(5) The following books are to be added to the Syllabus for the Intermediate Examination as far as applicable.

(1) Dacie—Practical Haematology.

(2) Mackie and MacCartney—Handbook of Practical Bacteriology.
(3) Harrison—Chemical Methods in Clinical Medicine.

Miss Scott stated that Nurses have a post-graduate Diploma, and thought we should have some higher qualification.

Dr. Fastier considered that the whole question of a Higher Examination would need re-analysing. What was to be the method of conducting the examination.

Would there be time to do the syllabus?

What will the standard be? If a thesis, would there be no written or oral exam? What would be considered a respectable thesis—it could be done possibly in Haematology, but examining would be difficult.

Mr. Ronald suggested we wait until the state of the final examination is clarified.

Mr. Jarratt: What is happening about the final examination?

Mr. Whillans: The Pathological Society Executive have that in hand, according to advice received from the Director-General.

Mr. Olive: "That in view of the opinion of the Pathological Society re the Higher Examination, action be deferred one year, the Council meanwhile to further consider the question." —(Olive-Adamson).

Moved.—"That a vote of thanks be passed to the Christchurch members for the very satisfactory Conference arrangements. —(Olive-Whillans).

Mr. Adamson, suitably replied.

Mr. Ellison expressed appreciation of the work of the Council, and of the conduct of the Conference.

The President declared the Conference closed at 5.30 p.m.

Numerous Papers were presented, also two films were show (Malaria, Terramycin).

On Saturday, 18th July, delegates were taken on a most interesting tour of Lincoln College.

The President expressed the thanks of the Association to all who had conducted members on the tour, and assured them that we had found much to interest us.

An innovation at the Conference in Christchurch this year was the holding of a Trade Display which was inspected by delegates during a half-hour allowed for morning and afternoon teas served in the display hall.

The active interest shown by all delegates as well as by heads of departments in the Hospital and certain Practitioners—all of whom were invited —was appreciated by the firms, all of whom expressed their satisfaction with the arrangements.

Anyone requiring any further information should write to Mr. J. J. Cannon, Pathology Department, Christchurch Hospital.

Details of Demonstrations and papers read at Conference, 1953.

(1) The Organisations of Work in the Auckland Hospital Laboratories. (Whillans).
(2) Physiological Aspects of some bacteria isolated from Clover Roots. (Miss Crozier).
(3) Salmonella infections of Animals in N.Z. (Josland).
(4) Rh. typing techniques. (Miss Scott).
(5) Immunity in Virus Diseases. (Dr. Fastier).
(6) A case of Monocytic Leucaemia. (Bridger).
(7) Demonstration of Specimens of Clinico-Pathological Interest. (Dr. Stewart).
(8) Public Health Laboratory Facilities from the M.O.H. Aspect. Dr. D. Kennedy).
(9) Direct Centrifuging of T.B. from C.S.F. on to Slide (Samuel).
(10) Conversazione and Demonstrations in Pathology Dept., Christchurch Hospital. (Path. Dept. Staff) .

Social.—A social function was held in the Students' Union Building on the evening of 17th July, and was an outstanding success, being thoroughly enjoyed by those attending.

USE OF THE PHASE CONTRAST MICROSCOPE.

F. J. Bergersen and M. N. Watt.

In an ordinary microscope the specimen to be viewed is illuminated at the apex of a cone of light and most of the rays that pass through the specimen are from the outer portion of this cone; the remaining rays, in the inner portion of the cone, form the background lighting. This fact is made use of in dark-ground, the central rays being blocked out leaving the background dark. The same principle was used by the older microscopists with Rheinberg's discs to obtain very beautiful colour effects with the low powers. This principle is the basis of today's phase contrast, but instead of altering the light waves before they reach the object they are modified after having passed through the specimen, which has been illuminated by a hollow cone of light as in dark-ground technique. Rays passing through the various parts of the object are altered in amplitude and phase according to the thickness and refractive index of those parts, but such alterations are usually not sufficiently marked as to be noticed by the eye. The intensification of these changes is the role of phase contrast. A diffraction plate is placed in the back focal plane of the objective; this plate is divided by an annular ring into three areas, that covered by the ring (the conjugate area), and the areas within and beyond it (together comprising the complementary area). The conjugate area is coated with a dielectric film that retards light waves, or a partially transmitting metal film that absorbs light waves, or both. The complementary area is similarly coated but with a different combination to the conjugate. Four combinations are therefore available, and the best to use for any particular subject can be found by experiment. The specimen itself, diffracts light in accordance with its structure, and this light passes mostly through the complementary area of the diffraction plate; the background light passes through the conjugate area only. Thus the diffraction plate may act differentially between the undeviated or background light, and the light deviated by the specimen. It will be seen that the light waves from the specimen, already altered in phase and amplitude by it, will be again altered in passing through the complementary area of the plate, waves coming together in phase will add up to increase brightness, while waves that are exactly out of phase and of equal amplitude will cause interference which the eye recognises as lack of light or black; waves of intermediate differences in phase produce a series of greys. The resulting image seen in the microscope is the sum of all the diffraction patterns forming it. The above is a very general explanation of how the phase microscope makes the detail visible.

The best type of equipment to use is that in which CONTINUOUS GRADATION from bright field, through various degrees of contrast to dark field illumination ,is obtained by means of a mirror component in the condenser.

Phase contrast microscopy will be found of great value in the differentiation of unstained tissues in the fields of histology, pathology

and botany; it will frequently differentiate tissues where staining will not give so clear a picture. In bacteriology its great advantage is the ease with which living material may be examined. Slide cultures of bacteria are easily followed in growth studies such as those of Fleming and co-workers and Pulvertaft, and one of us (F.J.B.) has used phase contrast observations of living cells in conjunction with the use of fixed and stained specimens in work on the cytology of bacterial growth. The examination of liquid cultures is easy and more definition is available than when using ordinary illumination, and motile bacteria, leptospira etc., are often more clearly seen by phase contrast than with good dark ground illumination.

Examination of histological sections, both stained and unstained is facilitated and such structures as the Golgi apparatus, have been noted to stand out clearly when observed with the phase contrast microscope.

Thus far the advantages described for this method of microscopy have been chiefly applicable to research problems but it is our opinion that the phase contrast microscope has a valuable contribution to make to routine diagnostic work, viz., in the field of parasitology, e.g., the structure of amoebic cysts is more easily determined than with any other method. Also, while the search for trichomonads in vaginal specimens is normally no easier than when standard methods are used, phase contrast illumination enable the determination of structure at a glance. This is of special value for specimens in which there are large numbers of pus and epithelial cells, when differentiation between parasite and host tissue is often difficult.

In the study of the structure of diatoms the use of phase contrast has proved a tremendous advance, not only are the specimens rendered more beautiful but resolution is increased to such an extent that it must be seen to be believed. The familiar test object, *Pleurosigma angulatum*, under phase contrast will show the complicated structure of the diatom using a 1/6in achromatic objective even better than the best 1/12in. oil immersion apochromat with ordinary transmitted light of shortest visible wave length. Further, phase contrast does away with the need of the special mounting media of high refraction index which have proved such a heart break to the earlier diatomists because of the tendency to crystalise in the course of time, thereby ruining many a valuable preparation.

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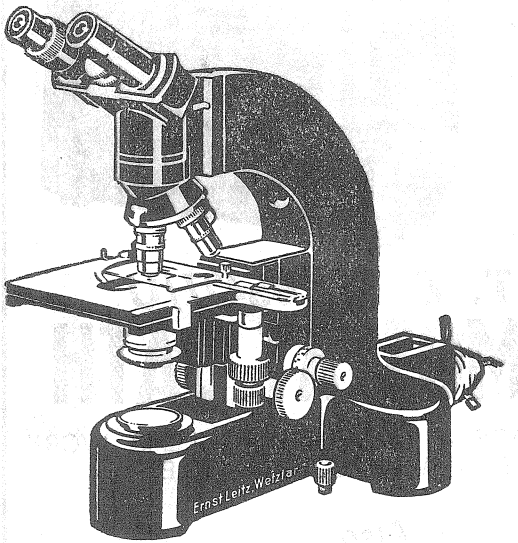

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