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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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Communications regarding this JOURNAL should be sent to the Editor, Department of Pathology, Greenlane Hospital, Auckland, S.E.3.

Communications primarily affecting the Association should be addressed to the Secretary, Mr. G. W. McKinley, Bacteriology Department, District Hospital, Waipukurau.

All monies should be paid to the Treasurer of the New Zealand Association of Bacteriologists (Inc.), Mr. H. T. G. Olive, Pathology Department, Public Hospital, Wellington.

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JOURNAL

of the

NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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EDITORIAL

With this issue the Journal enters a new phase. In the past it has been the sole responsibility of one person. The task has not been an easy one. Editing, printing, publishing and distributing have all been due to his industry and untiring enthusiasm, and now after five years it is only right and proper that the load should be distributed on a greater number of shoulders. We cannot adequately thank Mr. Whillans for his past efforts, and it is indeed fortunate that he is remaining on the editorial committee, where his advice and criticism will be invaluable.

There is one great problem, however, which cannot be solved by the setting-up of an editorial committee, and that is the providing of the material which constitutes the Journal. In this sense every member of the Association now has a greater responsibility, in that the printing is now being done by a commercial firm. If articles do not come to hand to warrant these greatly-increased costs, there can be only one outcome. Already the number of issues has been reduced from four to three per annum, and in future April, July and October will be the months of publication.

And so it is on this note that we begin our new life. We of the editorial committee feel that it would be a great pity if this valuable link between members were to die. It's up to you!

THE PLASMA CELL

J. H. Carter.

(Department of Pathology, Auckland Hospital.)

In 1890, while carrying out research work, Cajal observed a cell which he called the cianophil cell, a spherical or ellipsoidal structure varying in diameter from 7-14 mu. Its protoplasm was characterised by a deep-staining reaction with aniline dyes, and the presence of round vacuoles.

A year later Unna reported several of the same structures which he called "plasma cell," a name already used by Waldeyer (1875) to describe a non-related cell which also had a deeply staining basophilic cytoplasm. At this early stage Marschalks (1895) gave as a distinguishing characteristic of the cell the morphology of the nucleus, but stated that the basophilia of the cytoplasm was not specific. His theory was that plasma cells originated from tissue lymphocytes and that they were normal constituents of the connective tissue and not pathological.

Doan expressed the opinion that the cell arose from reticular cells. Further support to this statement was lent by Ching and Gordon (1942) and Cappell (1929). Lowenhauft, who has been conducting recent research, also favours a reticulo-endothelial origin. She noted that in multiple myeloma plasma cells were found in all lymph nodes. Plasma cells are distributed throughout the spleen and their occurrence there would be accounted for if they were of reticular origin. The conclusion that must be drawn from finding of lymph follicles intact, even with extensive plasma cell involvement, is that plasma cells are not of lymphocytic origin.

Plasma cells are formed when an endothelial cell divides in two planes forming a littorial cell and a plasma cell. They are classified on the basis nuclear, nucleolar and cytoplasmic characterists into plasmoblasts, early plasma cells and mature plasma cells.

The plasmoblast has characteristics of other primitive cells. It is larger than the mature cell and has a relatively round nucleus and a light blue unevenly staining cytoplasm. The nucleus has a delicate chromatin pattern and contains from one to four nucleoli. The nucleus tends to be eccentric, the nucleoli clear and well defined. The cytoplasmic margin is often irregular and transition forms can be found between this and the typical plasma cell.

The early plasma cell has a less distinct nucleolus than the plasmoblast, the nuclear chromatin is more compact, the cytoplasm stains a darker blue, the perinuclear zone is more distinct and the amount of cytoplasm in relation to the nucleus is greater. The cytoplasm stains unevenly and the shape of the cell is often irregular.

The mature cell is usually oval and is slightly larger than the neutrophil polymorph. The nucleus is relatively small, eccentric, round, and has a deep staining reaction.

The plasma cell varies in size from 7-14mu. The cytoplasm stains deeply basophilic using Leishmann, often staining more deeply at the periphery, forming a rim. In some of the cells the cytoplasm may stain so darkly that no structure is visible, but in the majority of cells the cytoplasm has a fine structure or shows bluish flecks. The cell is often said to have a granular cytoplasm, but granules as seen in neutrophil and eosinophil myelocytes are not found. In some cells the cytoplasm appears foamy or it may contain multiple vacuoles of various sizes.

The nucleus is usually eccentric, small, and exhibits a fine chromatin network in which there are occasional nucleoli stained a light blue. Sometimes the nucleus shows hypernucleation giving the appearance of a megakaryocyte.

The perinuclear region, or Area of Hoff, is a prominent, relatively unstained area near the nucleus. Theories regarding its structure and function vary considerably. Marschalks considered it to be an essential criterion of the plasma cell, while Joannovics and Schaffer said that the area represented a portion of the cytoplasm containing no basophilic substance. The most probable theory, that this region was the specific sphere of attraction of the cell, was proposed by Maximow and supported by Cajal, who demonstrated that this area contained a distinct Golgi apparatus. The appearance of this area, however, is inconstant.

Cytoplasmic vacuoles vary in number content and distribution. Some investigators interpret them as a degeneration phenomenon, while others regard them as temporary secretory cell states. By supravital staining with neutral red it can be shown that the vacuoles are not artefacts.

By staining the plasma cell with Pappenheim's stain, Russell Bodies may be demonstrated. These are spherical granules usually acidophilic but staining various shades. With this stain the bodies appear red in a background of green. They vary in size from eosinophil-like structures to large spheres of monocyte proportions. Russell Bodies were once regarded as pathological secretions but are now thought to be a degeneration phenomenon.

In normal bone marrow the plasma cell constitutes 1% of the leucocytes. It may occur in excess numbers in the peripheral blood in cases of infectious mononucleosis, cirrhosis, granulomas, measles, monocytic leukaemia, carcinoma, aplastic anaemia, chronic inflammations.

Several "speculations" as to the functions of the cell have been made. Jaonnovics and Schafer suggested that since plasma cells appear whenever there is destruction of nuclei their formation is due to local absorption of chromatin material. One quaint theory advocated by Bosselini (1902) was that the stainable material in plasma cells is nucleic and would be the basis of a new nuclei. A large group believed that these cells carried nutritive material. For some time the formation of these cells was inter-

preted as caused by an over-nourishment in connective cells. Still another theory is that the basophilic nature of plasma cells is due to an irritation in lymphocytes and that the cells are secretory corpuscles.

References:

The Plasma Cell. in Myeloma (Blood, 1948).
The Plasma Cell. (Michels, *Arch. Path.*, 1931.)
Parsons, J.: *J. Path. and Bact.*, 55, 397 (1943).
(1942) *Am. J. Med. Soc.*, 203, 829.
Lowenhauft (1939) *Quart. J. Med.*, 127.
(1947) *J. Lab. and Clin. Med.*, 32, 2.

(This manuscript was awarded first prize in the Technical section of the Essay Competition, 1950.)

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

PATHOLOGY DEPARTMENT, WELLINGTON HOSPITAL, FRIDAY, 27th, OCTOBER, 1950

Examiners: Dr. J. D. Reid and Mr. D. Whillans.

1. Write notes on the following terms:—
   Thermal death point;
   Antibody;
   Mean Corpuscular Volume (M.C.V.);
   The tungstic acid precipitation of protein.

2. Describe in detail how you would estimate the percentage of sugar in urine. (Illustrate your answer by supposing that the urine contains 2% of sugar.) What substances may give a false positive reaction?

3. Give briefly the postal regulations governing the transmission of pathological material by post.
   What specimens would be sent for the following examinations and how should they be packed:—
   (a) Blood for Widal,
   (b) Blood for malaria parasite,
   (c) Faeces for dysentery organisms.

4. Draw up a table giving the interaction of the serum and cells of all the blood groups AB, A, B and O. What do you understand by the terms "agglutination" and "rouleaux"? How would you differentiate between them?

5. How would you further investigate and confirm the identity of an organism suspected of being C. diphtheriae?

6. Describe how you would estimate the pH of a urine by an indicator method. What is the meaning of pH? What is the relationship between pH and titratable acidity?

PRACTICAL EXAMINATION

(3 hours)

Candidates will change places at three-quarter-hour intervals. Bacteriological examinations are to be completed from 9 a.m. on Saturday morning and the Oral portion of the Examination will be held in conjunction with this.

---

1. You are provided with specimens of a Gruel Test Meal. Perform a normal routine examination and graph the results obtained.

2. (a) Write brief notes on the solutions provided, mentioning briefly

   (i) their use,
   (ii) their composition,
   (iii) the value of and the reason for the component chemicals.

   (Esbach's Solution: Neisser Stain; Digestion Mixture: Solution for collecting blood for Transfusion.)

   (b) Identify organisms on the slides (a) to (d)—
   (a) from C.S.F.,
   (b) Urine deposit,
   (c) Throat swab,
   (d) from Robertson's meat media.
   (Meningococcus: Tubercle bacilli: Vincent's organisms: Tetanus.)

3. (a) Report on the four blood films provided.
   (Erythroblastosis: Lymphatic leukaemia: Stippling: Mononucleosis.)

   (b) Prepare a film, stain and perform a differential count on the routine blood provided.

4. (a) Make a complete routine examination, including culture, of the catheter urine provided.
   (Examination revealed pus, blood, Gram positive cocci, Gram negative bacilli.)

   (b) Examine the culture provided, identify the organism and determine its pathogenicity.
   (Staphylococcus aureus.)

CHRONIC PLASTIC BRONCHITIS

G. D. E. Meads

(Pathology Department, New Plymouth Hospital)

In the busy laboratory the worker is apt to conveniently push the careful macroscopic examination of the routine sputum aside. All may recognise at a glance the rusty specimen typical in pneumonia, or the bright blood suggesting tuberculosis, abscess or carcinoma. Do we take into account the amount of sputum, that pinkish, white, grey, salmon-coloured specimen with its possible indications? Does that foul odour have any clinical meaning to us? In neglecting the careful wet examination, are we missing those Charcot Leyden crystals, Curschmann's spirals or the possible fungi?

Plastic (fibrinous) bronchitis may be described as a rare and unimportant form of chest condition in which attacks of coughing accompanied by severe dyspnoea are associated with the presence in the bronchial tubes of tough fibrinous casts, which are ultimately coughed up with corresponding relief of the symptoms. The condition was apparently known to the ancients, but no satisfactory explanation has been found. It may be acute, but more often is of the chronic type and may continue for years. Mostly it is seen in young adults, but may be found in children and the old. Males are said to be more frequently affected than females (1). The diagnosis of plastic bronchitis is certain only by the presence of the casts. They may be surrounded by sputum so that they escape detection. The disease is to be suspected in patients who give a history of recurring attacks of paroxysmal cough and dyspnoea (2). A plug of sputum when unravelled and floated out in water reveals a cast of a part of the bronchial tree. The casts are usually compressed, chiefly of fibrin, but in some instances are of varying proportions of fibrin and mucus or mucus alone, as the main constituent. The number expelled varies from one every day or so to a large number daily. They may be in the form of a lump pellet or surrounded by sputum.

A typical case was recently seen in this laboratory. He was a man aged 74 years, who had been retired 10 years, after working for 15 years in a dusty fertilizer works. Up until six months prior to admission he had been taking half a fluid ounce of paraffin daily for many years, but had taken only three or four doses since then. Four months prior to hospitalisation patient had a "bout of 'flu" and began to cough up to ½ to 1 pint of sputum daily, which was occasionally tinged with blood. On examination the patient was slightly cyanosed and short of breath. There were minimal clinical signs in the lungs.

The provisional diagnosis was bronchitis or lipoid pneumonia.

Radiography showed some coarse mottled infiltration of the left and right lower lobes varying from day to day.

A routine blood count was normal, although the blood sedimentation rate was 45mms. in one hour.

Examination of the Sputum:

(a) Macroscopic.—The gross appearances was that of a clear lemon custard containing numerous large and small pinkish lumps and flakes somewhat resembling milk curd. When floated out in water and teased with a probe, these pellets revealed themselves to be unusually large and perfect bronchial casts. A single tree-like structure that covered a six-inch petri dish displayed more than twelve dichotomous branches. The smaller were solid, the larger hollow and showing on the outer surfaces the indentations of the bronchi in which they were formed.

(b) Microscopic.—The casts were found to be composed of fibrin, containing a few red cells, pus cells, many epithelial cells and masses of refractile globules which could be stained with Sudan III. A few pneumococci were seen. Films and cultures for M. tuberculosis were negative. The fluid portion with 1.88grams% of protein showed to a markedly lesser degree the cells and fat globules present within the fibrin cast.

The patient was started on a course of penicillin, which after eight days caused the main settling of temperature. After twelve days the temperature was completely normal, although the radiography showed no significant change and the patient still produced much sputum and many casts, the amount being slightly reduced.

The patient was finally discharged as chronic fibrinous bronchitis with congestive heart failure.

Discussion:

This is a case of plastic (fibrinous) bronchitis. It is rare and unusual in several aspects. The patient produced large amounts of sputum containing bronchial casts daily. These were expectorated with comparative ease and without the expected relief from dyspnoea.

The presence of fat globules presented itself as a problem without reasonable explanation, especially as they were contained almost wholly within the cast walls and had no resemblance to the fat globules of chyle micra.

Acknowledgments:

I wish to express my thanks to Dr. D. N. Allen and Dr. A. Veale for assistance and helpful criticism in preparing this paper, and to the Medical Superintendent for access to clinical notes.

References:

(1) Maurice Davidson (1935), *Diseases of the Chest.*
(2) Norris Landis (1938), *Diseases of the Chest.*

A VOICE FOR REFORM

J. Bridger.

(Department of Pathology, Public Hospital, Christchurch)

With five years of vigorous life planted squarely behind, the Association of Bacteriologists can afford to look ahead with confidence in their ability to meet the demands of the future.

The past is marked by a series of successful steps towards solidarity, comprising such advances as stabilisation of salaries, adequate representation to higher authority, a noteworthy—even if not, as yet, fully patronised—Journal, an Intermediate examination, and, latterly, the instituting of the half-yearly Finals.

The examinations themselves—both Intermediate and Final—are hedged at last by more or less clearly defined limits of experience and knowledge. But—and here there must, of necessity, be a significant pause—the method of arriving at these limits is still in a state of flux, and it is about this matter of training that I write.

It is a well-known but seemingly not fully appreciated fact that the prime factor governing the training of Bacteriologists is that there are a number of allied subjects and a certain standard to be reached in each of these.True, the accepted practice is to give personnel graduated lengths of time working with each subject, but is the time fully exploited regarding a composite theoretical ground? The answer is, I think, no.

There are many quite legitimate reasons for this regrettable lapse, and not the least of these is the time lack experienced by overworked departmental heads. But this is a perennial factor—the exigencies of practice versus the ideal. Even taking into account the clarion services of the departmental heads, there is still too much left to chance—in the form of the individual trainee's resolves—to make present conditions a satisfactory compromise. It might be satisfactory if the profession were stocked with real enthusiasts, combining an all-consuming interest and a high sense of moral responsibility both to themselves and to their chosen work. Unfortunately, or perhaps fortunately, no profession can lay claim to such a living ideal. We are human. We err, and, from time to time, our determination to take the most out of each and every part of the five-year course weakens.

If, then, we accept this existing state of affairs as fact, we must find an adequate remedy. The obvious solution must be an adaptation of present practice. At the moment, we rely on the individual to supplement work with theory in his own time, and this could continue—with an essential difference.

The Intermediate examination is the first and most important step in this direction, but it is not enough. The logical follow-up is interdepartmental examinations conducted within each hospital laboratory.

To elucidate with an example: A trainee spends three months doing Biochemistry. At the end of that time he is presented with a practical examination and a theoretical paper, the whole taking, say, three hours, to determine what his progress has been over the previous three months. A foreknowledge of this should serve to stimulate interest during that time and, if it does not, the onus is on the trainee concerned to repair the weakness.

Another and equally important benefit accruing from such a system is the experience it would give in working under examination conditions. Many a well-informed candidate has been nonplussed by the familiar presented in an unfamiliar fashion. Interdepartmental examinations should therefore serve as a refresher for the degree man and a prime necessity for the non-degree man. Viewed in this light, and three hours in three months—with perhaps an hour for discussion of the results—is, after all, not an exorbitant demand on the working time of either departmental heads or the hospital boards concerned. The time would surely pay for itself in every way.

A point arises here—and one well worthy of mention. It concerns the number and diversity of laboratory techniques in use in New Zealand to-day. These techniques are dependent on the individual experience and preference of senior personnel and pathologists, and naturally vary from place to place. Surely there is a need for a central record of current techniques other than between the pages of a dozen text-books. It is from such a record that the various departmental heads could draw, using its contents to pass on to their staffs and include in their three-monthly examinations. If this could be brought into effect there would be no necessity to crave the indulgence of external examiners in the matter of varying methods. The trainee would, in short, be completely up-to-date and informed, and it would be the corner-stone of his security for the future.

From all this one salient fact emerges. There can be no complete direction of training from any one central authority. It is, and—short of achieving University status—always will be a matter of local training. However, the basis of an improved training system could be initiated from such a central authority and backed by the previously mentioned record of techniques. Such an undertaking would be well in keeping with the progressive policy which five hard-won years have already fostered.

One final word: There is no claim to originality in any of the above ideas. They have, no doubt, echoed across the span of several years throughout New Zealand. There are places, moreover, where they are, in part, accomplished. But the part is not the whole substance.

This is, therefore, purely an attempt to present a picture of what the author deems to be the most pressing problem of the moment. Standardisation of training procedures is a necessity and must become fact before further demands can be made regard-

ing status, the higher examination and other allied projects.

If a platitude can be forgiven at this stage: we cannot expect to receive unless we are prepared to give requisitely, and nothing short of a thorough and, if needs be, expanded training schedule will suffice for the future and back the excellent work already done.

The way is open. Let the next step be firmly planted on that way.

(This manuscript was awarded first prize in the Literary section of the Essay Competition.)

ISOLATION OF INTESTINAL PATHOGENS

J. R. Callaghan.

(Department of Pathology, Auckland Hospital.)

The following is a summary of the intestinal pathogens isolated during 1950 in the Bacteriology Department of the Department of Pathology, Auckland Hospital.

For faeces, the routine followed was an initial plating on to Maconkey agar, plus enrichment in Selenite F medium followed by plating on to Maconkey agar.

For blood cultures, bile broth was used initially with plating on to Maconkey agar.

The antisera used were obtained from the Standards Laboratory for Serological Reagents, Colindale, London.

S. typhi

Total no. cases.	No. times isolated.	Isolations blood.	Isolations faeces.	Average isolations per case.
20	64	12	51	3.2

One isolation from infected ovarian cyst.

S. paratyphi A.

Total no. cases.	No. times isolated.	Isolations blood.	Isolations faeces.	Average isolations per case.
20	53	6	46	5.3

One isolation from gall bladder.

Salmonellae

Type.	No. of cases.	No. of times isolated.	Average isolations per case.
S. typhimurium	39	251	6.4
S. enteritidis	8	43	5.4
S. morbificansbovis	7	18	2.6

Shigellae

Type.	No. of cases.	No. of times isolated.	Average isolations per case.
Sh. flexner W	19	64	3.4
,, *88*	12	29	3.4
,, *Z*	1	3	3.0
Sh. sonnei	7	50	7.1

Total isolations	575
Total number of positive cases	123
Average isolations per case	4.7

HERE AND THERE

The Committees on Standard Methods and on the Higher Examination will meet in Wellington at the end of March, and will formulate a policy for dealing with all the methods sent in for approval, and such criticism as has been forwarded on the Higher Examination question.

Subscriptions are due and payable to the Treasurer, Mr. H. T. G. Olive, Department of Pathology, Wellington Hospital. Immediate attention to this matter will save him an immense amount of work. Senior £1/1/- and Junior 10/6.

The four successful candidates in the Intermediate Examination held in the Department of Pathology, Wellington Hospital, were Miss L. J. Gray, Kew Hospital, Invercargill; Miss L. E. Evans, Department of Pathology, Christchurch Hospital; Miss M. H. Smith, Bacteriology Department, District Hospital, Waipukurau, and Mr. A. Harper, Pathology Department, Palmerston North Hospital.

Miss M. H. Smith has now taken up a position with the Wellington Hospital in the laboratory so that she may finish her training there.

Mr. J. R. Callaghan, 1st Assistant Bacteriologist at the Auckland Public Hospital, and Mr. R. T. D. Aiken, Bacteriologist-in-Charge, Middlemore Hospital, have recently resigned from their respective positions and formed a partnership as Consulting Bacteriologists in Auckland.

Mr. P. H. Curtis has also recently resigned from the staff of the Auckland Hospital Laboratories. He is going to work for Dr. Lindsay Brown, formerly of Cornwall Hospital, who has gone into private practice as a Pathologist.

It is with much regret that we report the death of Mr. Len Haden, Bacteriologist at Whangarei Hospital. One of the oldest members of our profession, Mr. Haden trained first in the Army during the 1914-1918 war and then under Mr. Armitage in the old Public Health Laboratory in Auckland. He had served Whangarei Hospital for more than twenty-five years.

Mr. M. Jenner, formerly of Dunedin, is now working in the Pathological Department, Christchurch Public Hospital.

There was an interesting report of the Council of Clinical Pathologists (Eng.) on the supply, demand and training of laboratory technicians in the English National Health Service in the Year Book, 1949.

It gave an appreciation of the skill and initiative required in a good laboratory technician, and mentioned the close inter-relationship of Pathologist and Technician in the work of the laboratory. It also praised the work of the Institute of Medical Laboratory Technology and declared it to be worth all encouragement.

It mentioned the present universal shortage of trained staff consequent on the advances in laboratory medicine, and favoured the retaining of the apprenticeship system rather than the shortening of training time in schools of laboratory technique, as well as the need to keep the salary level attractive.

The demand in England was probably, in all, about 5,000, but the wastage of women by marriage would eventually even itself out as the proportion of trained men increased. The supply of applicants was excellent, although the proportion of girls to boys was rather high.

In training there was the preliminary training in the laboratory with educational night classes at the local night schools. It had been found that the educational authority tends to overload the trainee with theory, whereas the prime reason for the technician is his technical ability. There

was also the approved laboratory where roster systems of internal departments under full-time Pathologists takes place, and it was suggested that trainees should only be registered at such a laboratory.

It is interesting to see that whereas they have previously gone in for specialist examinations, it is suggested that perhaps in the future the general examination in clinical pathology will be the qualifying examination.

Of further interest is the fact that they disapprove of any method of "clocking-in," as in medical work there must be overlapping of work schedules, and nothing must be done to destroy the technicians' pride of work.

Mr. L. B. Fastier, of the Virus Research Laboratory, Medical School, Dunedin, gave a very interesting talk at the last Conference in that city. He touched on the future of virus work both here and in the United States of America, and referred us to his paper in the N.Z. M. Journal, April, 1950, XLIX, 140.

He mentioned the economic factors in the setting-up of a virus laboratory, such as the use of much expensive equipment; the quantity of equipment used (e.g., syringes); the costly space used to separate all departments of such a laboratory, such as serology, egg inoculation and autopsy; the price of laboratory animals such as mice and ferrets; the cost of fertile eggs (at least 7½d per egg), and went into the question of tissue culture.

Of those virus infections identified by laboratory service he mentioned:

(a) The Pneumotropic (1) Upper respiratory (influenza, cold),
(2) Lower respiratory (Viral pneumonia, Psittacosis, Q-fever.).

Diagnosed either serologically or by inhibition of haemagglutination.

(b) Dermotropic: Measles, chicken-pox, smallpox, herpes.

(c) Viscerotropic: Yellow fever, infective hepatitis, serum transmitted jaundice.

(d) Neurotropic: Encephalitis, encephalo-myelitis, poliomyelitis, all encephalitides (the latter very infectious).

In sending specimens to him he recommended that the specimen be suspended in 50% glycerol in saline of pH 7.2. The specimen should be sealed carefully with paraffin wax over a tight cork and packed in much cotton wool to prevent accidental breakage, the cotton wool being itself soaked in formalin. The specimen then loaded into a thermos packed in crushed ice and dispatched to him air freight, first sending a telegram giving the exact time of arrival of the plane in Dunedin. He also warned listeners that the Laboratory in Dunedin was purely a Research Unit and could not undertake routine Virus Diagnosis, though it was hoped to prepare satisfactory antigens there for routine use in New Zealand.

He finished by describing the new Virus Research Laboratory being built at the moment and was accorded a hearty vote of thanks for his interesting and informative lecture.

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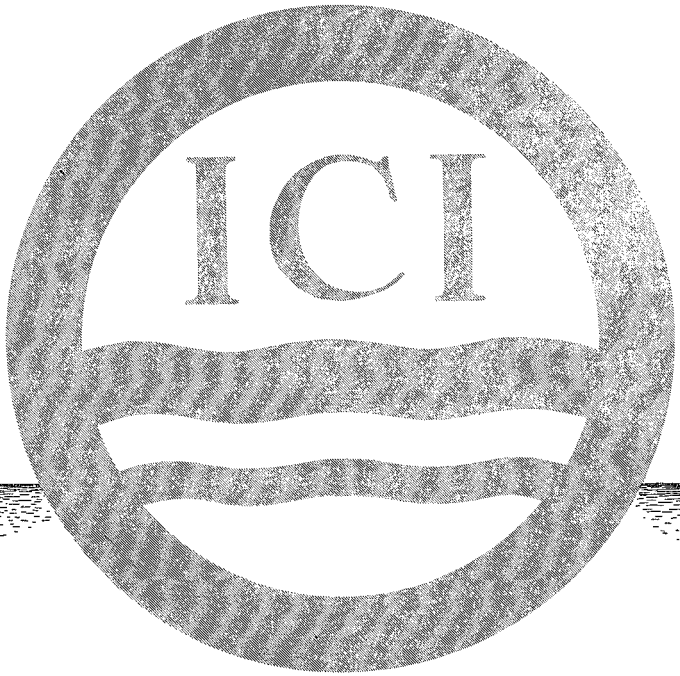

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