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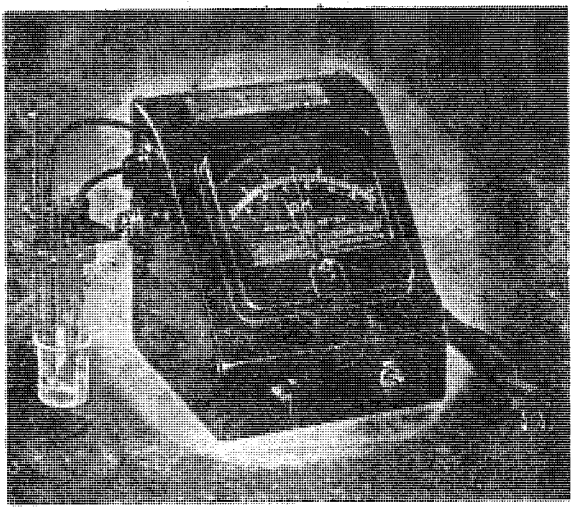

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THE ISOLATION AND THE DETERMINATION OF SENSITIVITY TO STREPTOMYCIN OF MYCOBACTERIUM TUBERCULOSIS.

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Mycobacterium tuberculosis was shown by the masterly research of Robert Koch in 1882 to be the causative organism of tuberculosis; not only did he demonstrate the bacilli in the lesions and culture them on an artificial medium, but he was successful in animal transfer from pure cultures, thereby fulfilling his own postulates.

The type of solid medium used by Koch for primary isolation was inspissated blood serum, and the fluid medium he used in 1890 for the production of tuberculin was a 5% glycerin bouillon. There was practically no substantial contribution to the knowledge of the growth requirements of the tubercle bacillus during the subsequent twenty-five years, then gradually the addition of egg substance, potato, salts and inhibitory dyes to a nutrient base was introduced. It was, however, the discovery of antibiotics and their application in tuberculosis which greatly stimulated the production of adequate laboratory methods for the isolation and cultivation of *M. tuberculosis* from pathogenic material.

In 1912 Rappin [1] announced that the filtrates of cultures of *B. subtilis*, *B. megatherium* and *B. mesentericus* strongly inhibited the growth of *M. tuberculosis* in vitro. This apparently original work in the discovery of tuberculostatic agents "in vitro" was to remain without addition until January, 1944, when Schatz, Bugie and Waksman announced that they had obtained an antibiotic substance they called Streptomycin from the fungus *Actinomyces* (*Streptomyces*) *griseus*. In November, 1944 Schatz and Waksman reported that steptomycin exerted a bacteriostatic effect on a human strain of M. tuberculosis.

Youmans reported in 1945 that streptomycin was bacteriostatic at a concentration of 0.095-0.28 units per cc. and bactericidal at a concentration in excess of 50 units per cc. It is important that the unit of streptomycin is understood. The Penicillin unit or Oxford unit is based on inhibition of *S. aureus*

in 50 cc. medium, Streptomycin is defined in terms of its action against the gram negative *Escherichia coli* in 1 cc. medium. The Oxford unit represents a much greater "in vitro" activity than does the streptomycin unit. In terms of dry weight 1 streptomycin unit equals 1 microgram, whereas I Oxford unit of penicillin equals 0.6 microgram.

One of the greatest disappointments encountered in the streptomycin treatment of tuberculosis is the frequent tendency of the tubercle bacilli to become resistant to teh drug when therapy is prolonged. Fisher (2) reported that tubercle bacilli were capable of developing resistance to streptomycin following either "in vitro" or "in vivo" exposure to the antibiotic. Since the therapeutic value of streptomycin for tuberculosis may be greatly influenced by this development of resistance, it soon became important to determine the degree of development of bacterial resistance. Filoman, Kerlson and Henshaw (3) reported that infections in guinea pigs induced by tubercle bacilli resistant to streptomycin were refractory to treatment with this antibiotic. Goodacre, Mitchell and Seymour (4) reported on the tuberculostatic behaviour of a compound para-amino salicylic acid (PAS) in low concentration "in vitro." Bloch, Vennestand, Ebert and Gomori (5) then showed that the combination of streptomycin and PAS had a much more favourable effect on experimental tuberculosis in the guinea pig than either drug alone. Yegian and Vanderlinde (6) have recently stated that *no reports have cited the development of strains resistant to the combination of drugs* when both drugs are given in combination to a patient who has never previously had either drug. These authors stress that streptomycin-resistant tubercle bacilli are rapidly becoming the predominant type in certain patient populations and suggest that in time they may replace the original parent type cells. Recently reports have been made of patients who appear to have developed their disease as a result of infection with highly resistant tubercle bacilli. It thus should be the policy to test routinely the streptomycin sensitivity of the tubercle bacilli from all patients with tuberculosis presumably acquired since 1947. Valuable information concerning the epidemiology of the disease may be gained *now* if this study is made before the number of patients infected with streptomycin-resistant organisms constitutes the majority. Knowledge of the streptomycin sensitivity of the tubercle bacilli in new cases is of importance with regard not only to subsequent treatment but also to the source of the infection.

As streptomycin became more readily available in New Zealand, the importance of reviewing our existing diagnostic methods in the light of recent advances in the bacteriological techniques associated with the primary isolation and cultivation of M. tuber-

culosis became obvious. In order that clinicians should receive the maximum assistance possible in relation to the streptomycin therapy of pulmonary tuberculosis this review was undertaken. The following is a brief summary of current knowledge and the advances which have been made during the last few years in the isolation, cultivation and determination of streptomycin sensitivity of *M. tuberculosis*.

Cultivation of *M. tuberculosis* from contaminated pathological material can best be accomplished if proper methods of digestion and decontamination are used; cultivation on artificial media is slow and difficult and requires special methods to prevent the multiplication of contaminating organisms commonly found in specimens from which tubercle bacilli must be isolated. It is essential that the methods employed to reduce contamination do not retard growth of the tubercle bacilli. However, as yet no satisfactory method has been devised for the concentration of faeces.

Despite the many improved methods which have been suggested in cultural techniques, it is not always possible to initiate growth in cultures from microscopically positive specimens. Preparation of specimens for smear, culture or animal inoculation by digestion and concentration necessitates exposure of the material to various more or less toxic substances. The purpose of such treatment is to dissolve tenacious material and cellular debris and to kill secondary organisms.

Corper and Nelson (7) showed that the original sodium hypochlorite method introduced about 1908 appeared to be as reliable for detecting tubercle bacilli as any of the more complex procedures, including the use of Tergitol, a new organic wetting agent introduced in 1939 as a digestant. Until in 1946, when Corper and Stoner (8) described the use of trisodium phosphate in preparation of sputum for diagnostic culture of mammalian types of *M. tuberculosis*. These workers reported that this organism was not destroyed by contact with a 10% solution (23% $Na_3PO_412H_2O$) of trisodium phosphate for a week at room temperature and that contaminating organisms in sputum were destroyed without hazard to the tubercle bacilli by contact for 24 hours at 37°C.

Spendlove, Cummins and Patnode (9) in a survey of digestants showed that Sodium Hydroxide and Trisodium phosphate were less toxic than oxalic acid, sulphuric acid, hydrochloric acid or ammonium carbonate; they also showed that infectivity of tubercle bacilli in most sputa was lost after 4 days at 37°C.

Various other authors (10) have proved trisodium phosphate provides better results than any other known digestant although sulphuric acid remains the most powerful disinfectant towards

other germs.

Schwartung ([11]) and others have shown that inhibitory gastric lavages were acid and usually contained germicidal factors, and that the bactericidal effect is directly related to the length of time and the height of temperature at which the lavages are kept. Kabler and Lundholm ([12]) confirmed this observation and showed that the addition of crystalline $Na_2PO_412H_2O$ soon after collection to the specimen of gastric juice resulted in a considerable increase in positive cultures from specimens sent by mail.

The method of obtaining and treatment of the centrifuged deposit of the concentrated spemimen before insemination has also been investigated. Weiland ([13]) claimed that the use of a high speed centrifuge doing 10,000 r.p.m. increased the percentage of positive smears from 7% in a control group to 14% at high speed; also positive cultures obtained were 17% in control group and 22% at high speed. Lockard ([14]) advocates the use of 250 ml. centrifuge bottles plus rubber stopper and centrifuging at 2000 r.p.m. Other authors have shown that washing the centrifuged deposit repeatedly with saline before insemination reduces the viability of *M. tuberculosis* and the best method after concentration is that recommended by Gissens-de-Casol ([10]), the deposit being washed with Sorensen buffer ph 7.0 twice before planting. Others recommend the addition of 200 units of sodium penicillin per 1 ml. of deposit.

Many investigators ([15]) have attempted to obtain information which might aid in the identification of potential anti-tuberculous agents by determining the bacteriostatic effect of compounds on the tubercle bacilli "in vitro." A wide variety of media have been used: (a) Complex nutrient media; and (b) synthetic nutrient media. The latter probably should be called "chemically defined" media, since all the constituents are known. Type 1 media consist, when used in the solid form, of a nutrient base to which is added egg yolk, glycerol and often a variety of other ingredients. Lowenstein and Petragnani medium are two common examples which have the overall disadvantage of being of unknown chemical composition, making it impossible for them to be duplicated accurately. Type 2 or chemically defined media on the other hand are easily prepared and can be duplicated readily. The rate of growth of tubercle bacilli on these media can be determined and is fairly constant.

In 1945 a new medium was described by Dubos ([16]) which was reported by him to produce rapid growth of the tubercle bacilli. This Tween-albumin liquid medium permitted the production of young and homogenous cultures of the submerged sediment type which were sufficiently well dispersed to allow their manipulation by the ordinary bacteriological techniques. The

dispersed growth was obtained by the effect of non-ionic wetting agents, the poly-oxyethylene esters of sorbitan monostearate (Tween 60), sorbitan monooleate (Tween 80). Corper, Cohn and Frey ([17]) showed that a concentration of 1% or lower of Tween 80 was non-toxic to tubercle bacilli.

The Dubos and Davis liquid Tween albumin medium is reported ([18]) as being ideal for determining streptomycin sensitivity of virulent human or bovine tubercle bacilli and the effect of its various constituents has been accurately determined. It was found that Tween 80, even in low concentration, increased the sensitivity of the majority of strains to streptomycin. Serum (bovine) increased the rate of growth and decreased slightly the streptomycin sensitivity of some strains. Glycerol increased slightly the resistance of a few strains of tubercle bacilli to streptomycin. In the presence of serum, however, glycerol had no observable effect. It was also found that within reasonable limits the amount of inoculum employed did not effect the degree of sensitivity to streptomycin.

It has been proved that turbimetric measurements in Tween-albumin medium provide an accurate method of measuring growth of *M. tuberculosis* in the presence of streptomycin. The results of streptomycin sensitivity tests show that the sensitivity of a culture of tubercle bacilli to streptomycin "in vitro" is partially dependent on the composition of the culture medium employed. Thus the ideal medium would be one which duplicated precisely the conditions which obtain "in vivo" and was itself really and accurately reproducible.

In the past, the fraction V of bovine plasma has been used almost exclusively as a source of albumin. Unfortunately this material is costly and not readily available to workers outside the U.S.A. However, Dubos, Fenner and Pierce ([19]) have recently devised a method whereby human serum conveniently and inexpensively can be prepared to act with the same growth-promoting properties as the fraction V. Cultures of tubercle bacilli grow rapidly and diffusely in a liquid medium containing this crude albumin fraction of human serum and a small amount of the wetting agent Tween 80.

Giammolio, Nalsios and Elton ([20]) have adapted the technique of Pryce and Rosenberg for the rapid cultivation of *M. tuberculosis* on a glass slide in a laked blood medium containing known concentrations of streptomycin, and it affords a simple and apparently effective method for rapid determination of the sensitivity. The advantages of this method lie in (a) the availability of the medium and (b) the approximation of the medium to a natural milieu and (c) the finesse of the end-point afforded by microscopic reading.

Youmans, Ibrahim, Sweaney and Sweaney ([21]) have recently suggested that the employment of a solid culture medium might permit the determination of sensitivity at the time of primary isolation, by direct inoculation of concentrates of sputum or other pathologic material on to the streptomycin-containing medium. If feasible, this method should furnish a more exact evaluation of streptomycin resistance than using transplanted pure cultures, because nearly the total bacterial population of each patient's sputum is thus represented. Furthermore, the time required is no longer than that required for primary isolation of the tubercle bacillus.

Having reviewed recent work from which our methods were adopted, it remains briefly to record these methods and the first statistical results associated with their use. It is not suggested that these methods are the best or most accurate, but that they are regarded as most suitable to the resources of this laboratory.

The Streptomycin Sensitivity of M. Tuberculosis.

Laboratory Procedure.

Isolation of Organism:

Sputum from cases of pulmonary tuberculosis when received were poured into sterile bottles and an equal amount of 10% Na_3PO_4 solution was added. If the sputum was unusually heavy and mucopurulent it was found necessary to use more digestant. The bottles were stoppered, shaken and placed in the incubator (37.5°C.) for 18-24 hours. The bottles were then removed from the incubator, 1 drop of 0.1% phenolphthalein was added, and 2NHCl was added dropwise until the treated sputum was colourless. The specimen was then transferred to the largest available sterile centrifuge tube and centrifuged at 3000 r.p.m. for 20 minutes. The supernatant fluid was decanted, leaving a few drops to facilitate mixing the sediment. The sediment was then divided into two portions, one being cultured immediately on to a tube of Petragnani medium using a wireloop and flaming before and after each inoculation. The other portion of sediment received five drops (0.25 cc.) of a solution of sodium penicillin 1000 units/cc.. This was thoroughly mixed and a second tube of Petragnani medium was then inoculated. The remaining sediment was smeared thickly on a glass slide, dried, heat fixed and stained according to Ziehl-Neelsen method. Results of the miscroscopic examination were incorporated in the records. The cultures were incubated at 37.5°C., were examined weekly and were not discarded until after at least eight weeks' incubation. When typically dry, crumpled, raised, yellowish colonies of *M. tuberculosis* were observed on the egg medium; a large loopful

representative of the growth was emulsified in 0.5 ml. of distilled water. After the grosser particles had been allowed to settle, about 0.2 ml. of the suspension was transferred to a tube of the Tween-albumin medium which was incubated for about 7-10 days. when there was usually a good diffuse growth.

Medium:

The synthetic medium of Dubos and Davis was prepared as follows:—

Potassium dihydrogen phosphate (KH_2PO_4)	1.0 gm.
Disodium hydrogen phosphate ($NaHPO_4 12H_2O$)	6.25 g.m.
Sodium Citrate	1.5 g.m.
Magnesium sulphate ($MgSO_4\ 7H_2O$)	0.6 g.m.
Dissolved one at a time in glass distilled water.	
Add Tween 80 (a 10% solution)	5.0 c.c.
Casein hydrolysate (a 20% solution)	10.0 c.c.
Glass distilled water to	1000 c.c.

The medium should have a pH of 7.2. It was distributed in 4.8 c.c. amounts and autoclaved at 10 lb. pressure for 10 minutes. A 10% solution of bovine albumin (fraction V, Armour & Co.) was prepared, Seitz-filtered and 0.15 c.c. added before use to each 4.8 cc. medium, giving a final concentration of 0.3% bovine albumin when the volume in each tube was made up to 5 c.c. The Casein hydrolysate was prepared as for casein yeast agar (Mackie and McCartney, 1942).

Sensitivity Test:

0.1 c.c. of the 7-10 day smoothly growing culture in Tween albumin medium was inoculated into a series of four tubes of the same liquid medium containing concentrations 1.0, 10 and 100 micrograms of streptomycin per /cc.—the fourth tube acting as a control. The dilutions of steptomycin to give these concentrations were prepared from a stock solution of 1% streptomycin which was prepared freshly every month. The various concentrations of streptomycin were prepared so that a volume of 0.05 cc. when added to the tube of medium gave the necessary units of antibiotic and completed the volume to 5 cc. medium. The series of tubes were incubated at 37.5°C. and were examined at frequent intervals for 14 days. The sensitivity was read on the fourteenth day and was expressed as the lowest concentraion of streptomycin per cc. giving complete inhibition of growth.

It was possible to estimate the relative number of resistant organisms in a culture by observing the lag period before growth appears. In other words, a culture which produced growth in

the tube containing 100 units of streptomycin per cc. in four or five days would be considered to contain a majority of organisms resistant to 100 units, whereas a culture which showed growth on this tube only after 12-14 days of incubation could be considered to have less than 1% of its population actually resistant to 100 units per cc. of medium. This observation was confined to the laboratory. Until sufficient practice is obtained in making readings, no reports of percentage resistant forms will be issued. Only the actual sensitivity of the strain as read on the fourteenth day was reported.

Observations and Results:

On the basis of both theoretical considerations and practical experience there appears to be good reason for classifying as significantly resistant those strains of *M. tuberculosis* which grow in culture media containing 10 units streptomycin per cc. A very impressive correlation has also been observed between the incidence of resistant tubercle bacilli and the type of tuberculosis being treated.

It is essential to adopt a safety technique in pipetting tubercle bacilli in this test and the operation should only be entrusted to qualified bacteriologists.

Tubercle bacilli that were found to be resistant to streptomycin "in vitro" were found to retain this characteristic for at least six months when cultured in the absence of the drug.

More positive cultures were obtained from the solid medium inoculated with the penicillin treated sediment than from the untreated sediment. The streptomycin sensitivity of stains isolated by both methods from the same sputum showed no variation.

In the small group of sensitivity tests reported here, all except one of the patients from whom the strains were isolated had received a course or courses of streptomycin of 1 gm. per diem in two doses of 500 mgm. twelve-hourly. The sputum from the one patient receiving no streptomycin gave a normal sensitive strain on isolation. Comparison between the results obtained in our first small series of sensitivity tests and the much larger series of Blattberg and Ehrhom ([22]) and Wolensky ([23]) show a surprising degree of correlation.

This first series of patients tested were those who had not received treatment with PAS at the same time as streptomycin. It is hoped later to review a series of patients receiving combined PAS and streptomycin therapy.

Summary:

An evaluation of recent advances in laboratory procedures for the isolation, cultivation and determination of sensitivity to Streptomycin of Mycobacterium tuberculosis has been given. A method suitable for a general hospital laboratory has been tried

and the results obtained from a small series have been compared with those obtained by research workers.

Acknowledgment:

I am indebted to Dr J. O. Mercer, Pathologist and Acting Superintendent-in-Chief of the Wellington Hospital, for permission to publish this paper.

Table of Sensitivity Results.

Patient No.	Total Streptomycin Received, 1 gm. per diem 500 mgm. b.i.d.	Units of Streptomycin per c.c. 1	10	100	Control		Reported Sensitivity. Units Streptomycin per c.c.
1	51	—	—	—	**		1
2	49	—	—	—	**		1
3	41	**	**	**	**	Insen	100
4	59	—	—	—	**		1
5	96	**	—	—	**		10
6	39	*	—	—	**		10
7	50	**	**	**	**	Insen	100
7	47	—	—	—	**		1
8	47	—	—	—	**		1
9	48	—	—	—	**		1
10	35	—	—	—	**		1
11	86	**	**	**	**	Insen	100
12	26	*	—	—	**		10
13	139	**	*	—	**		100
14	150	**	**	**	**	Insen	100
15	46	—	—	—	**		1
16	43	**	—	—	**		10
17	119	—	—	—	**		1
18	97	**	**	**	**	Insen	100
19	—	—	—	—	**		1
20	64	**	**	**	**	Insen	100
21	25	**	—	—	**		10

— No growth. **Heavy growth. * Appreciably less growth than control.

Table of Percentage of Resistant Strains Produced in Relation to Period of Streptomycin Therapy.

Streptomycin Days of Treatment.	This Series.	Blattberg and Ehrlom (22)	Wolinsky (23)
30	10%	No resistant forms.	10%
60	35%	13%	39%
90	45%	29%	42%
120	50%	45%	
150	60%		

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Abbreviations: ART = The American Review of Tuberculosis and Abstracts. AJCP = The American Journal of Clinical Pathology. JLCM = Journal of Laboratory and Clinical Medicine.

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MEDITERRANEAN ANAEMIA—COOLEY'S ANAEMIA

N. J. Ellison, Wellington.

In 1889 Von Jaksch described an anaemia associated with enlargement of the spleen and leucocytosis to which he gave the name "Anaemia Infantum Pseudoleucaemia." As time went on a miscellaneous group of similar conditions was included under the name of Van Jaksch's syndrome. In 1921 Cooley reported an anaemia associated with splenomegaly in two patients, brother and sister, of mongoloid appearance and showing distinctive skull changes. In 1925 Cooley and Lee clearly separated this particular disorder from Von Jaksch's complex syndrome. The condition was variously described as Cooley's anaemia, erythroblastic anaemia, Thalassemia, and Mediterranean anaemia. It showed an haemolytic dysfunction quite distinct from congenital haemolytic jaundice and the sickle anaemia of the negro. Cooley himself preferred the name of "erythroblastic anaemia," but later developments have shown that this term was confused with "erythroblastosis foetalis." Because of the predilection of the disease for children of Greek, Italian, Syrian, or Armenian parents, "Mediterranean Anaemia" was suggested. However, relatively recent reports show that the condition is not entirely confined to people in the vicinity of the Mediterranean coast.

The disease has a wide range of clinical severity from a mild form, which may not be apparent, to a severe form. The case that I describe belongs to the severe group and, so far as I know, is the only recorded case in New Zealand. The child is a female, the only child of Greek parents.

Symptomatology.

The onset was characteristically insidious. At four months of age the child manifested the first noted evidence of the disease.

The parents said that the child had become lethargic and was refusing to take food. A physician was consulted and he noted the slight muddy colour and pronounced anaemia. The spleen was enlarged and possibly the liver. There were no glands apparent and no obvious jaundice. At four months of age the blood picture was as follows: Red count, 3 millions/cmm. White count, 17,800/cmm.; Hb, 8 grams; Polys, 25%; Lymphos, 73%; Mononuclears, 2%.

Stained film showed hypochromia, poikilocytosis, microcytosis, and the presence of "target cells." The notable feature, however, was the substantial number of nucleated red cells with normoblasts predominating.

Many blood counts were carried out, but I will not weary you with them all and have selected a few representative ones.

Fairly intensive iron therapy was prescribed for the child.

Three weeks later the blood picture showed no improvement and there was a larger proportion of nucleated red cells present. Reticulated cells numbered 12 per cent. The fragility test normal.

Blood counts and fragility tests carried out on the parents were normal.

At six months of age the spleen had enlarged a good deal more and liver enlargement was then obvious. There was still no evident jaundice.

X-ray of the chest was clear and no glands were apparent.

The red cell count was still 3 millions but the haemoglobin had dropped two grams to 6 grams. The leucocyte count was 30,000. The stained film showed the characteristic "target cells" and many nucleated red cells normoblasts still predominating. Again the fragility test was normal.

A transfusion of 240 ccs. of Group O Rh negative blood was given.

Five weeks later the red cell count was the same and the haemoglobin content had increased by nearly two grams to 7.7 grams. Nucleated red cells numbered 75 per 100 white cells. (Total white count 12,000.)

At this stage (the child was then just over 7 months of age) a sternal puncture was performed. The bone marrow showed erythroblastic hyperplasia and 70 per cent. of the nucleated red cells were normoblasts largely immature. There was no increase above normal in myeloid cells.

At the age of 8 months, i.e., two months following the transfusion, splenectomy was performed. Just prior to surgical procedure the red cell count was nearly 3 millions, haemoglobin 6 grams—a decline of nearly two grams in three weeks—Leucocyte count 20,000. Nucleated red calls numbered 80 per 100 white cells. Reticulocytes numbered 12 per cent. and the fragility test was again normal.

The spleen weighed 8oz. and was enlarged and soft. The greater part of the tissue was occupied by rather diffuse foci of erythropoiesis with many reticulum cells and cells showing stages of development towards mature normoblasts. The sinuses were distended with similar cells. There was no evidence of leukaemia. The appearances were those of marked splenic erythropoiesis which whilst not diagnostic of, were consistent with, Cooley's anaemia.

A fortnight after splenectomy nucleated red cells numbered 100 per 100 white cells (Leucocyte count 16,000).

A month later 167 per 100 white cells (Leucocytes 19,000).

Two months later nucleated red cells numbered 300 per 100 white cells (Leucocytes 21,000). Platelets numbered 16,200.

At the age of 13½ months, i.e., five months after splenectomy, there were 500 nucleated red cells and 100 leucocytes (white count 30,000, Red cell count 2 millions, Haemoglobin 6 grams). Incidentally the count from the white cell pipette was in toto 200,000 per cmm.

Rays of the skull, long bones, hands and feet were carried out at the age of 9 months. The findings suggested, but were not diagnostic of, Cooley's anaemia.

Further X-rays at 13 months of age showed changes in the frontal bones of the skull consistent with Cooley's anaemia. The trabeculation of the leg bones was coarser than normal but was not sufficiently definite to be significant.

The child at the age of 13½ months had a more obvious "muddy" colour and presented a definite mongoloid appearance.

As I mentioned earlier, the cases of Cooley's aneamia recorded fall into mild, moderate, and severe forms. The clinical, haematological, and radiological finds, I think, place this case in the severe group.

The exact cause of the disease is obscure. Exhaustive studies by a number of observers have shown that Cooley's anaemia is due, not to lack of extrinsic iron (the case described did not respond to haematinic therapy) but to an inherited inability of the body to utilise or synthesise some substance essential to normal erythropoiesis. Efforts to identify the missing factor have so far been unsuccesstul. According to this concept the difference between mild and severe forms would be a matter of difference in the capacity of the body to elaborate the unknown deficiem substance.

The early enlargement of the spleen is probably due to its excess erythropoiesis and overactivity in disposing of the products of fragmentation of the red corpuscles, and the liver enlargement is no doubt associated with the persistent anaemia.

It appears that the only function that splenectomy performs is merely that of comfort by the absence of abdominal discomfort.

Fundamentally the diagnosis of Cooley's anaemia in the severe state depends on the following:

(1) Splenomegaly.
(2) Anaemia with the characteristic red cell picture of erythroblastosis.
(3) Resistance to antianaemic aids.
(4) Distinctive skeletal changes.

And of lesser importance,

(5) "Muddy" colour and mongoloid appearance.

The presence of pigment in the tissues is considered to be an error in metabolism and rather resembles that found in haemochromatosis. This latter condition, however, rarely shows anaemia.

The absence of crises differentiates the condition from that of congenital haemolytic jaundice. There is little doubt that Cooley's anaemia has an heritary origin, even though the severe cases usually fail to reach adolescence, and those who have gone on to maturity have failed to procreate. The question that arises is not *whether* it is transmitted but *how?* I do not propose to bore you with the many theories that have been propounded but will merely state that Cooley himself believes that the inheritance is by a simple dominant and leave it at that.

With regard to prognosis, individuals suffering from the mild form may, and usually do, have a normal span of life, whereas those who develop the severe form, with early onset in life, marked anaemia, enlarged spleen, and bony changes, rarely survive adolescence. They succumb from profound anaemia or intercurrent infection.

To any student of haematology the study of this syndrome is an all-absorbing one no less interesting than that relatively recent entity erythroblastic foetalis.

(Lantern slides in colour of these blood films were shown during the course of this paper, which was delivered at the sixth Annual Conference, 1950.)

THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS.

Minutes of the Sixth Annual Conference held at the Medical School, Otago University, on 17th and 18th August, 1950.

The President introduced Sir Charles Hercus, Dean of the Medical Faculty, and Professor D'Ath, Professor of Pathology, Otago University, and mentioned that they were distinguished Honorary Members of the Association, and examiners for the final examination. As Sir Charles Hercus had to leave for the North in a short time, he proceeded with his address immediately.

Address:

Sir Charles Hercus, D.S.O., O.B.E., B.D.S., M.D., D.P.H., F.R.A.C.P., F.R.A.C.S., said that it was a privilege and a pleasure to address the Conference and welcome them to the Medical School.

He regretted that he was leaving almost immediately on a tour of the northern faculties, as this would prevent him from sitting in at the Conference as an Honorary Member.

He expressed his astonishment at the growth of Medical Science, as represented by the Association, over the last 20 years, and recalled the days when there were less than 20 persons engaged in laboratory practice, while now the Association had an active Membership of 160. The phenomenon of the century was the rapid march of Medical Science, and the Medical School illustrated this development.

The new South Block was a monument to the progress of Medical Science, and typified the growth of the Medical School.

Sir Charles drew the attention of delegates to the sculpture on the Hanover Street panel at the entrance to the South Block, representing the "spirit of Medical Service," and reminded them that the members of the Association were engaged on such a service to mankind, and he asked

them to note well the words inscribed, "Observe—Remember—Compare."

He spoke in detail of the special facilities embodied in the South Block, and described the various departments, especially Bacteriology, Pathology and Microbiology, and told of the astounding growth of all branches of work. He pictured the whole as a "Research Institute," composite and embodied within the University, and mentioned that among the special laboratories were the N.Z. Medical Council Research Laboratory, the British Empire Cancer Campaign Laboratory, and the Travis Research Laboratory for Tuberculosis, and in connection with the last named the speaker expressed his opinion that a cure for tuberculosis will come from careful research on the nutritional needs of T.B.

The experimental Animal section was considered to be a very good department and delegates would find much to interest them there.

In conclusion, Sir Charles invited those present to see all facilities and move freely about the Medical School and absorb the spirit of service embodied in the University, and in welcoming delegates to Dunedin he expressed the wish that they would have a successful Conference and an enjoyable stay in the University city.

The President thanked Sir Charles Hercus and Professor D'Ath for their courtesy in attending our Conference and placing at our disposal the facilities of the Medical School.

Sir Charles Hercus then withdrew, to commence his journey North.

Conference Opened:

Professor D'Ath said that it was very gratifying to see so many present, especially the older Bacteriologists, and in particular he mentioned Mr. Pierard, of Wellington Hospital, who had taught him 35 years ago.

The speaker recalled the early days of Laboratory practice, and said that the younger people had to thank the older generation of Bacteriologists for the training they had given.

The Professor said that he would like to see first-rate facilities for instruction in laboratory work, and he realised we had that as a common aim.

After inviting us to look around the Medical School and ask all the questions we could, Professor D'Ath said that it gave him great pleasure to declare the 1950 Conference open and wished the Association every success.

The President thanked Professor D'Ath for declaring the 1950 Conference open, and said that we welcomed the opportunity of seeing for ourselves the work that was being done at the Medical School.

Professor D'Ath then withdrew, as other urgent business claimed his attention.

The President's Address:

On behalf of the Council of the Association I extend to you all a very hearty welcome to the Sixth Annual Conference.

It is most gratifying to see such a large and representative gathering here to-day. There are present members from as far north as Whangarei, and it is with some feeling of pride that I am privileged to occupy the Presidential Chair.

The past year has been an eventful one. With extreme distaste your Association was embroiled in litigation earlier this year. You are well aware that certain members, if not all, of our profession were in danger of being incorporated in the Hotel Workers' Union. Thanks to our solicitor and to representatives of the Association who appeared in Court on our behalf, the danger was averted, and I am sure it will not face us again. I would like placed on record our appreciation of the splendid work of Messrs. Olive, Schwass and Rolet, who so ably spoke on our behalf during the Court hearing.

Once again the Council has functioned smoothly and I thank my colleagues for their willing help and co-operation. Without prejudice, special credit must go to our competent Secretary, Mr. McKinley, and Treasurer, Mr Olive. Both have had an exceptionally busy year. For our Editor-in-chief-cum-Printer, once again I have nothing but the most profound admiration. The time has come, however, when some of the load that he has borne for so long must be lifted from his shoulders.

In conclusion, I have to tell you that Mr. Samuel and his colleagues have a treat in store for you all, and I take this opportunity of thanking them for the splendid arrangements for the Conference programme and for the accommodation of the visitors.

May I wish you one and all a most happy and instructive Conference.

The President then mentioned that Miss Partridge, of Wanganui, had been ill for a considerable time, and the Conference directed that the Hon. Secretary write to Miss Partridge expressing the Conference's best wishes for a speedy recovery.

Roll Call:

The following delegates were present at the opening of the Conference, and several members of the Medical School departments attended at times when their duties permitted:—Miss J. Mattingley and Messrs N. J. Ellison, H. T. G. Olive, J. Pierard and F. Austin (Wellington), Mr. G. W. McKinley (Waipukurau), Miss J. Byres and Messrs. D. Whillans, R. Aitken, P. Curtis and A. M. Murphy (Auckland), Misses M. Taylor and L. Evans and Messrs. F. Corey, J. Murray and D. H. Adamson (Christchurch), Mr. K. B. Ronald (Oamaru), Mr. E. L. F. Buxton (Wanganui), Mr. H. Ward (Timaru), Misses B. Broughton, J. Penrose and L. Boock, and Messrs. L. B. Fastier, D. Bacon, M. Jenner, M. Morris and F. Kershaw (Dunedin), Messrs. I. W. Saunders and B. Meade (New Plymouth), Mr. G. George (Rotorua), Mr. S. O. Jarratt (Palmerston North), Mr. C. G. Thompson (Invercargill), Mr. V. J. Hawke (Nelson), Mr. S. W. Iosland and J. J. G. Peddie (Wallaceville), Miss F. Macdonald (Ashburton), Mrs. Moroney (Hastings), Miss C. Saxby (Napier), and Misses E. Wylie, H. MacDiarmid and A. Simmonds (Hamilton).

Apologies:

Miss Richards, Mr. Ekdahl, Mr. J. Smith, Mr. W. Carruthers, Mr. J. Callaghan, Mr. A. F. Bell, Miss Patrridge, Mr. H. Hutchings, Mr. M. Keenan, Miss M. Smith, Miss B. Smith.

Proxies:

No proxies were declared, no doubt due to the operation this year of postal voting for the election of officers.

Remits:

No remits were forwarded to Conference, 1950.

Minutes of 1949 Conference:

The Minutes had been published in the "Journal" and were taken as read.

Moved "that the Minutes of the 1949 Conference be confirmed" (Josland—Pierard).—Carried.

No points were raised for discussion.

Annual Report and Balance Sheet:

This was presented by the Hon. Secretary and Hon. Treasurer, having previously been made available to delegates.

"That the Annual Report be adopted." (Whillans—Buxton).—Carried.

"That the Balance Sheet be adopted" (Jarratt—Murray).—Carried.

Honoraria:

Mr. Editor, £20; the Hon. Secretary, £3/3/-; the Hon. Treasurer, £3/3/-; the Auditor, 1949, £2/2/-; the Auditor, 1950, £2/2/-. (Buxton—Jarratt).—Carried.

Scrutineers:

The Conference confirmed the action of the President, Secretary and Treasurer, who were all unopposed, in acting as scrutineers. (Byres—Buxton.)—Carried.

Election of Officers:

Office-bearers for the year were elected as follows:—President, Mr N. J. Ellison (Wellington); Vice-Presidents, Mr. E. L. F. Buxton (Wanganui) and Mr. D. Whillans (Auckland); Hon. Secretary, Mr. G. W. McKinley (Waipukurau); Hon. Treasurer, Mr. H. T. G. Olive (Wellington); Members of Council, Miss J. Byres (Auckland), Mr. D. H. Adamson (Christchurch), Mr. S. O. Jarrett (Palmerston North), and Mr. A. Samuel (Dunedin).

Moved: "That the Hon. Secretary destroy the ballot-papers." (Olive—Jarratt.)—Carried.

General Business:

The Essay Prizes.—The 1950 Essay Prizes were announced, the successful entries being:

The Plasma Cell.—J. H. Carter, Auckland.

A Voice for Reform.—R. Bridger, Christchurch.

Mr. Bridger was a previous winner in 1947.

The President asked that the Council compile a list of suggested subjects for Essays.

Mr. Whillans asked that the Council should consider a proposal to notify the Department of Health of the names of new trainees.

Mr. Murray asked that the Council should make inquiries re the application of the Basic Wage.

At this stage the Hon. Secretary reviewed the year's work of the Council, for the information of delegates. Briefly, the main points were as follows:—

The Department of Health: Pre-requisites for the Final Examination:

At present the University Entrance, or School Certificate plus the Intermediate examination, is required.

The candidate must have completed five years' laboratory training, and the two years following the intermediate examination must be in a base hospital Laboratory.

The Department has authorised attendance at Conferences on the lines circulated to Hospital Boards. This will be subject to approval each year.

Examination Dates and Certificates:

Examination dates and the venue will be notified to all hospitals three months before the date of examination. Results will also be notified to the Association.

The Intermediate Certificate is now a worthy recognition for those who pass this examination.

A mid-year final examination will be held if at least four candidates are offering, or if there are special circumstances as determined by the Pathologist's Advisory Committee.

The final Certificate is being brought into line with the format desired by the Association, and will no doubt prove acceptable to all members.

The Salaries Advisory Committee:

The Secretary outlined the Council's activities in full, and Amendment No. 15 was gazetted on the 26th July, 1950, as members know.

The Director-General of Health advised that under the new procedure of the S.A.C. persons may appear before the S.A.C. to state their individual case, and then withdraw. The general increase in salaries on the 8th May, 1950, is being examined by S.A.C. with regard to its possible application to Laboratory workers.

Meeting with the Pathologists:

Mr. Whillans and the Hon. Secretary met the Conference of Patholo-

gists in Auckland in May, 1950, and were well received. In general the submissions of your delegates have been met in full.

Points raised were:—

(1) The Association's pending (at that stage) legal action.
(2) The clarification of the pre-requisites for the final examination.
(3) The form of the final Certificate.
(4) Institution of a Higher Examination (after the finals).
(5) A policy of training, and the Senior Syllabus.

Your delegates consider the liaison established with the Pathologists and the Department of Health at this yearly Conference is of great value to the Association.

The Association v. the Hotel, Restaurant and Related Trades Employees' Industrial Union of Workers (Inc.):

The Secretary dealt in detail with the whole case, and emphasised that it was the *unanimous* decision of the Council that this action must be fought and won, as it was not considered that the interests of the Association would be served by allowing to pass unchallenged the possibility that at a later date members of the Association would be compelled to join the above Union. The preliminaries and subsequent Court action extended over a period of nine months, from the opening moves to the delivery of judgment.

The Association's case was upheld by the judgment of Mr. Justice Hutchinson.

A full report of the case has been published in the journal "New Zealand Hospital," Vol. 3, No. 1, p. 3, September, 1950.

Examinations and Successful Candidates During the Past Year:

Final Examination, February, 1950.—W. E. Browne, P. H. Curtis, C. E. Felmingham, H. E. Foster, Miss M. K. North, Miss S. Perl, F. M. Rush-Munro, Miss C. Saxby.

Intermediate Examination—October, 1949.—Miss J. M. Bailey, Miss K. R. Biggs, Miss B. L. Broughton, J. W. Carroll, Miss M. M. Dick, C. E. Felmingham, Miss E. I. Hicks, J. T. Holland, H. E. Hutchings, Miss H. J. Macdiarmid, G. R. C. Meads, F. D. Mulligan, Miss M. K. North, R. J. Patterson, W. J. Sloan, J. P. Walsh.

May, 1950.—Miss M. Armstrong, J. T. Connolly, M. M. Donnell, D. J. Philip, Miss S. A. McFadgen, I. R. Buxton, A. L. Schwass, F. L. N. Corey, Miss D. E. Sewell, F. C. Kershaw.

The President said he was sure that Conference would join with him in extending congratulations to all successful candidates for examination in the past year.

The Journal:

Mr. Whillans, retiring Editor, traced the history of the Journal, which commenced with 12 pages a quarter and is now 32 pages a quarter. Mr. Whillans has been Editor, printer and publisher for five years, and suggested that the Journal be printed commercially three times a year.

The President and several other speakers all paid tribute to the magnificent work done by Mr. Whillans over the last five years, and regretted that for reasons of health he must give up much of his "Journal" work. The Association was indeed greatly indebted to Mr. Whillans, and he could be proud of the standard of the "Journal" from the first issue onwards.

Election of Editor:

Mr. A. M. Murphy. (Whillans—Olive)—Carried.

It was moved "that the 'Journal' be printed thrice yearly." (Olive—Murray)—Carried.

It was left in the hands of the Editorial Committee to act in this matter.

The Higher Examination:

The Executive of the Pathological Society had suggested to the Association that a Higher Examination, say a Fellowship, be conducted by the

Association. Alternatives were suggested, and the Council on 16th August, 1950, recommended the adoption of one of these, namely, an examination in one branch of Laboratory Practice, to be taken a minimum of three years after passing the final examination.

The Secretary opened the discussion by making it clear that the proposed examination was an academic qualification, and that it was not intended that the higher qualification would carry any monetary benefit, but that those holding this examination would probably be in a better position to rise to the more senior positions in the profession, which, incidentally, were more highly paid.

Mr. Fastier wondered what were the advantages. The medically qualified man would be the specialist. He personally was not actually against the idea, and considered that as a means of enabling members to work with a definite aim after the final examination, the proposed examination could serve a useful purpose.

Miss Byres supported the idea, and pointed out that the Association was interested in raising the standard of the profession, and that money was not the only consideration, or even the main one.

Mr. Jarratt: Is it officially recognised?

The Hon. Secretary: As the suggestion has now come from the Executive of the Pathological Society, I presume the Department of Health will be in accord with the idea.

Mr. Whillans: The Pathologist is in charge of the work, which is chiefly carried out by the Hospital Bacteriologists and trainees. If the standard is to progress, staff must be trained and the best methods used. He supported the idea of a Higher Examination and suggested a thesis as part of the requirements.

Mr. Buxton and Mr. Jarratt both spoke in support of the proposed examination.

Mr. Ronald asked what was the fellowship and membership standard in England.

Miss Byres outlined the English scheme.

Mr. Whillans pointed out that this had been published in the "Journal."

Mr. Saunders: What ideas have we for preparing candidates for this examination?

Mr. Olive: It will be open to any qualified person who has been through finals for three years. Candidates must do their own preparation.

Mr. Murray would like to see a worth-while standard adopted. For those attempting the examination in biochemistry he considered a M.Sc. degree a pre-requisite. Who would examine candidates?

Mr. Ellison: We may control the examination, but Association members need not be the examiners.

Mr. Jarratt: In any event the Association will assuredly demand a high standard.

Mr. Ellison: Well, it is up to us. We must move forward, and overcome difficulties if they arise.

Mr. Fastier said he was not against the idea but he wanted recognition. He accepted gladly the suggestion from the Pathologists and wanted to see a high standard, but thought that external examiners would be the right idea.

Mr. Whillans hoped the examination would come to pass and would be a forerunner of better things.

It was moved: "That a high qualification be instituted on the lines of (c) in the Pathological Society Executive's letter, and consideration be given to incorporating a thesis as part of this examination."—(Jarratt—Olive)—Carried.

Mr. Murray suggested that the Council and co-opted members should proceed in this matter. Members could send in their opinions to the Hon.

Secretary, and perhaps any concrete proposals could be circulated to laboratories for comment.

Method of Voting:

Miss Byres appealed for preferential voting.

After much discussion it was decided that the special committee appointed last year should investigate the possibilities of this method.

The minutes of a Special General Meeting held at Wellington Hospital on 26th November, 1949, having been published in the "Journal," were taken as read.

"That the minutes be confirmed." (Buxton—Olive)—Carried.

Venue of Conference, 1951:

It was decided that Conference, 1951, be held at New Plymouth, after Mr. Saunders had assured us that we would be welcomed in Taranaki.

(Buxton—Olive)—Carried.

The General Meeting closed at 3 p.m. with a vote of thanks to the chair.

Presentation of Papers and Demonstrations:

The programme followed that was circularised to all members prior to the Conference.

The Papers presented were:

Mediterranean Anaemia—N. I. Ellison; Viruses—L. R. Fastier; Sterilisation by Steam—D. Whillans; The Frog Pregnancy Test—S. O. Jarratt.

Mr. R. Aitken added interesting special angles to Mr. Whillans' paper.

Demonstrations:

Demonstrations were set out in Room 221, which was open throughout the Conference.

(1) Pathogenic Fungi Isolated in New Zealand, and Scheme for Identification. Dr. M. J. Marples and Miss M. DiMenna.
(2) Viruses. L. B. Fastier.
(3) Grouping and Typing of Haemolytic Streptococci. Dr. L. Kirschner.
(4) Microculture of T.B. and Fluorescence Microscopy. J. A. Samuel.
(5) Freeze-drying Apparatus for Stock Cultures and Centrifrugal Freeze-drying Apparatus. J. A. Samuel.
(6) Apparatus Making for Greater Efficiency in the Laboratory.

These are listed in greater detail elsewhere.

Building and Laboratory Design:

Symposium on Building and Laboratory Design. Introduced by J. A. Samuel and D. Whillans.

Tour of the new South Block, demonstrating overall design, heating and ventilation and steam generation by automatic electric plant, lighting and acoustics, unit biological and chemical laboratories and special facilities for Bacteriology and Pathology.

Mr. Adamson and Mr. Murray also spoke on Laboratory design.

The papers presented will appear in the "Journal" in due course.

The Conference is greatly indebted to a willing Committee of Dunedin people who had worked so hard to make the Conference a success.

On the final evening of the Conference a cocktail party was held at the University Club Rooms, and was an undoubted success. This social function filled a need wihch has been apparent at all previous Conferences, that of providing time for delegates to exchange ideas informally.

Those fortunate enough to be present at the 1950 Conference considered that a very high standard was reached and that there was much to be learned from the Medical School Laboratory, Otago University.

Exhibits at the 6th Annual Conference of the New Zealand Association Bacteriologists.

1. Lamp for transilluminating and warming rabbit's ear for intravenous injections.
2. Design for rabbit-box (for holding when injecting intravenously).
3. Detoxification and concentration of urine for pregnancy tests. (Adsorbing hormones on Kaolin at pH 4.0 and eluting hormones into solution at pH 9.) (Scott, L. D.: 1940 Brit. J. Exp. Path., 21, 320.) (Notes on Male Test for Pregnancy. Law. Bul. Institute Med. Lab. Tech., Vol. 14, No. 11, Sept.-Oct., 1949.)
4. Jewellers' lenses which can be used on spectacles.
5. Tungsten carbide pencil for marking and numbering glass.
6. Survey of airborne molds in Dunedin district. Plates were shown which had been exposed under standard conditions at different places in the district. Also cellophane disc method for obtaining large quantities of molds for extraction and use for desensitisation.
7. Pathogenic fungi in New Zealand. Exhibits were: The Identification of Candida albicans, Cultures of *Trichophyton Mentagrophytes* (fluffy and powdery), *Trichophyton (achorion) schoenleini and rubrum, Microsporum audouini, gypseum and canis; Epidermophyton flocossum; Candida (monilia) albicans; Cryptococcus neoformans (Torula histolytica)*. Miscroscope slides of *Candida albicans chlamydospores.* Slide culture of *Trichophyton mentagrophytes (fuseaux and favic chandeliers)*. Slide culture of *Microsporum canis (fuseaux)*. Slide of *Epidermophyton floccosum (fuseaux).* Special Media for Isolation and Identification of Fungi: Sabouraud aga pH 5.5 and pH 4.0; 2% sugars; Littmans' oxgall crystal violet agar; Cornmeal agar and extract; Dextrose veal infusion; Dextrose yeast extract broth pH 4.0.
8. *Release of Jammed Syringes*:
   (a) Fill dead space in syringe with water, using small syringe and needle.
   (b) Make combination needle by forcing point of 22-gauge needle into 19-gauge needle.
   (c) Fit one end of needle on to jammed syringe and fit tuberculin syringe full of water on other end.
   (d) Steady push on plunger of tuberculin syringe forces fluid between barrel and plunger of jammed syringe, lubricating and freeing the plunger and forcing it out. This method never fails or breaks a syringe.
9. 1/7th-inch oil immersion objective for TB searching and differential counts.
10. Multiple counter for differential counts.
11. *Watson Victor exhibits* Watson "Kima" microscope stand; Sartory Petri dish searcher; Terry anglepoise lamp; Watvic microscope lamp; Burton Fresnel miscroscope lamp; Schillabers immersion oil, grade A and B; Abbe camera lucida; Horstmann Pluslite (illuminator and magnifier); Hanovia model ultra violet fluorescent lamp; 500 ml. Isomantle (for heating flasks of inflammable liquid); Gowlands lens stand and lens; illuminated magnifier; two types interval timer; Sunvic hot wire switch; Sunvic adjustable thermostat; Qualtex constant temperature hot water bath; Watson "Bactil" microscope.

13. Freeze dryer using Edwards speedivac pump attached to multiple tube manifold. (Drying agent in manifold $CaSO_4$.) Pirani vacuum gauge; Centrifugal freeze dryer for drying large amounts of serum or liquids. Edwards pressure and vacuum pump.
14. Chance Flamemaster blow-torch with multiple jets, straight and cross fire.
15. Glass tube cutter, using red-hot wire in close contact with tube. Wire heated by 13-volt 10-amp. circuit.
16. Photographs of remodelled Christchurch laboratory.
17. Numerous arrangements of time-clock switches and micro-switches.
18. Acoustics of lecture theatre using cross-section of room in ripple tank.
19. Cleaning egg or Loeffler slopes by soaking in 10% tri-sodium Phosphate and 10% Teepol for one week.
20. *Virus Research* Exhibit: High-speed centrifuge head for attaching to ordinary centrifuge.
21. Elford ultrafilter.
22. Marconi pH meter.
23. Microscope preparation showing inclusion bodies.
24. Plastic visor for protection against splashing in virus fluids.
25. Diagram showing methods of using chick embryos for virus studies.
26. Diagram showing culture of influenza and rickettsiae.
27. Yolk sac from 8-day chick embryo.
28. Carrel flasks for tissue culture.
29. *T.B. Detection and Culture*:
    Showing pros and cons of egg medium tubes and ordinary methods of detection, and also of suggested methods of growth in fluid egg yolk medium and detection by fluorescence microscopy. Slides were shown of growth after certain given times by ordinary microscopy and fluorescence microscopy. Light filter, both substage and eyepiece, were also shown.
30. Method of separating egg yolk from yolk sac.
31. Dust-proof slide-holder.
32. Device for demonstrating colony appearance by transmitted and reflected light, using fluorescent tubes.

Minutes of the Council Meeting held in the Medical School, Otago University, on 16th August, at 7.30 p.m.

There were present Mr. N. J. Ellison (President and Chairman), Messrs. Buxton and Whillans (Vice-Presidents), Mr G. W. McKinley (Secretary), Mr. H. T. G. Olive (Treasurer), Miss J. Byres, and Messrs. D. H. Adamson and S. O. Jarratt (Council Members). There was an apology from Mr. M. O. Ekdahl, who has recently taken up his appointment in Gisborne.

Mr. A. J. Samuel (Otago Medical School) was present by invitation.

The Hon. Secretary was instructed to write to the Salaries Advisory Committee asking that the salary for University Entrance first-year trainees be raised to £195-£215 per annum; that those people covered by the Hospital Employment Regulations, 1948, 1950/126, Amendment No. 15, should receive the basic adult wage on reaching the age of 21 years, and advising that Messrs. H. T. G. Olive and H. Ward were the Association nomina-

tions for S.A.C. deputies.

He was also instructed to write to the D.G.H. informing him that the Association will investigate the proposal to introduce standard methods. (See elsewhere for Committees.)

He was further instructed to write to the Secretary of the Pathological Society asking (1) for clarification of the proposal that there be an addition to the syllabus for the Intermediate Examination in the fields of chemistry, anatomy and physiology; (2) that the present final examination be retained with a higher standard and that a higher examination be taken not less than three years after qualifying for the final examination.

The following new members were admitted to the Association: Misses D. Strain and J. Penrose, and Messrs. J. D. R. Morgan and M. Jenner (Dunedin), Miss I. Jarmolicz (New Plymouth), Miss H. Schreuder (Gisborne), Miss A. Simmonds and Messrs. M. Harper and B. Barry (Waikato), Miss D. Diggle (Westport), Miss M. Lindsey (Masterton), Miss McCallum and Messrs. W. Don and D. G. Till (Christchurch), Mr. K. G. Reeve (Wanganui), Mr. J. Hughes (Auckland), and Mr. J. J. Cannon.

The following resignations were received with regret:—Miss M. Barnett (Dunedin), Miss L. Williamson (Christchurch), Miss M. North (Palmerston North), and Miss S. Kirkland (Dannevirke).

Mr. Whillans reported that after printing the October, 1950, issue of the Journal he would have completed five years' work of editing, printing and publishing for the Association. He regretted that it would not be possible to continue owing to the strain, but offered to do job printing for the Association. A committee will be set up to deal with the publication of the Journal.

The remainder of the meeting was then spent in arranging the last-minute details of the Conference. The meeting concluded at 12.25 a.m.

COMMITTEES ON STANDARD METHODS

Following representations of the Director-General of Health, the following sub-committees have been framed to work on Standard Methods. These committees must report to the Hon. Secretary of the Association by December 31st, 1950. The person named as convenor will be responsible for seeing that the committees act forthwith, and will forward the findings to the Hon. General Secretary.

Sterilisation: D. Whillans (convenor) and R. Aitken.

Bacteriology: S. O. Jarratt (convenor), D. H. Adamson, A. Samuel, L. Reynolds, F. M. Rush-Munro.

Haematology: E. L. F. Buxton (convenor), I. W. Saunders, I. M. Cole, and Miss P. B. Scott (Grouping and Rh).

Biochemistry: H. T. G. Olive (convenor), J. T. Murray, Miss S. Perl.

Miscellaneous (Histological technique, animal inoculations, frog and other tests for pregnancy): D. Whillans (convenor), J. Cole, S. O. Jarratt, L. Reynolds.

COMMITTEE TO INVESTIGATE THE INSTITUTION OF A HIGHER EXAMINATION

The President and the Hon. Secretary (ex officio), J. T. Murray, L. Reynolds, I. W. Saunders, D. Whillans.

The President will act as convenor of this committee, which is required to report to the Hon. Secretary of the New Zealand Association of Bacteriologists by January 31st, 1951.

Members of the Association are invited to forward ideas on this proposed Examination to the Hon. Secretary of the N.Z.A.B.

PATHOGENIC FUNGI KNOWN TO OCCUR IN NEW ZEALAND

Notes on the Pathogenic Fungi Exhibit.

Species.	*Lesions Caused.*	
	Causing Superficial Mycoses:	
Trichophyton mentagrophytes ..	Tinea pedis, "athlete's foot"; tinea cruris; tinea; barbae sometimes tinea capitis, corporis and unguium (ringworm of the scalp, body and nails).	Mycelium and spores in skin and hair, not characteristic of the species, but hairs infected by *Microsporum sp.* and by *T. schoenleini* show green fluorescence under a Woods lamp. *E. floccosum* never invades the hair.
Trichophyton (Achorion) schoenleini (No published report of its occurrence in N.Z., but it has been isolated in the Medical School on three occasions.)	Tinea capitis, corporis and unguium.	
Epidermophyton floccosum	Tinea pedis, tinea cruris.	
Microsporum audouini	Tinea capitis.	
Microsporum canis (lanosum)..	Tinea capitis, tinea corporis, occasionally tinea pedis and tinea cruris.	
Microsporum gypseum (fulvum)....		
Malassezia (Microsporum) furfur)	Pityriasis (Tinea) versicolor.	Characteristic arrangement of spores and mycelium in skin. Growth does not occur on the usual media.
	Causing Superficial or Deep Mycoses:	
Candida (Monilia)	Moniliasis of nails and interdigital clefts, vulvovaginitis and thrush. Rarely, meningitis, pulmonary lesions and monilial granulomas. Present, in small numbers, in the normal flora of mouths, faeces and vaginae of some individuals.	Oval yeasts, with or without mycelium in skin, anal and vaginal swabs, sputa, nail clippings and C.S.F.
	Causing Deep Mycoses:	
Cryptococcus neoformans (Torula histolytica)	Torulosis of C.N.S., pulmonary lesions, localised skin infections.	Spherical yeasts with a wide capsule. No mycelium at any time.
Actinomyces israeli	Lesions of head, neck, thorax and abdomen; other sites less frequently.	Fine, twiggy mycelium, sulphur yellow macroscopic granules are diagnostic but are not always present.
Actinomyces bovis	Actinomycosis of cattle.	Fine twiggy mycelium.

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POSITION VACANT.

A qualified Hospital Bacteriologist is required for a private laboratory doing general Pathology in Hamilton. Enquiries may be addressed in the first place to the Editor of this Journal, stating age and experience.

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