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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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JOURNAL
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New Zealand Association of Bacteriologists

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EDITORIAL

With this issue a new era starts for the Journal. After ten years' meritorious service Auckland members have retired. May we say thank you to the people concerned. We hope to do as well but emphasise that any publication must rely on its contributors for its excellence. A mystical approach to an abstruse subject cannot pass for quality. Matters which are everyday problems produce the most constructive material for articles. Hand in hand with quality must come at least some quantity. This is where the greatest difficulty is encountered and it must be overcome if the Journal is to qualify for respect among periodicals of a similar nature. One member of the Association has objected to the practice of publishing the minutes of Association conferences. In answer to this we would say that the number of technical articles generally available now would not of themselves justify publication of the journal. However, the responsibility is his and yours to produce enough material to warrant continuation. Every Bacteriologist must have ideas and experience which can benefit others. Reluctance to bring these forward will help retard the progress of the Journal. Please help.

"PIGMENT PRODUCTION BY A STRAIN OF PSEUDOMONAS AERUGINOSA ISOLATED FROM THE FLEECE OF SHEEP"

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(Canterbury Agricultural College)

INTRODUCTION

Pseudomonas sps. are widely distributed in nature (Breed et al. 1948), however systematic relationships of related organisms are as yet in many cases poorly characterised, and differentiation is often difficult. Because of the ease of identification of pyocyanine pigment produced by typical strains, *Ps. aeruginosa* is among the easiest of bacteria to identify. Atypical strains which fail to produce this pigment are common, however, and have been described by Jordan (1899), Gessard (1919), Lehmann et al. (1931) and Gaby (1946). Pyocyanine producing strains have been found to lose the capacity to produce this pigment, and identification of such strains depends largely upon their history of having once been able to produce pyocyanine, or, upon the fact of their isolation from pathogenic processes in higher animals. Organisms identified as *Ps. aeruginosa* have been isolated from a variety of other sources including soil, water, sewage, normal skin and from the fleece of sheep. Slimy or mucoid strains of *Ps. aeruginosa* are seldom encountered under these conditions but are usually associated in the main with pathogenic conditions.

A second pigment characteristic of *Ps. aeruginosa,* retaining its chromogenic function, is a non-specific water-soluble fluorescent pigment which is yellow-green to green by transmitted light and highly fluorescent in ultra-violet light. Jordan (1899) suggests that *Ps. fluorescens* might be a degenerate form of *Ps. aeruginosa* and this theory is also contributed to by Sandiford (1937), Gaby (1946) and Christie (1948). However, the opinion of those unwilling to admit the existence of other distinct species is expressed by Stanier (1947), who suggested the term "Pseudomonas fluorescens species group" for fluorescent pseudomonads unable to manufacture accessory phenazine pigments.

Early work has shown that pigment production is inconsistent (Gessard 1892, Thumm 1894, Jordan 1899, Eisenburg 1914) as various cultural methods caused *B. pyocyaneus* to form fluorescent pigments alone, pyocyanine alone or both pigments together. Baerthlein (1918) obtained variants containing no pigment, the fluorescent pigment alone, or both pyocyanine or fluorescin, from a single culture. Because of the variability in pigment production much attention has been given to the specific nutrient elements necessary for the production of one or other of these pigments or

both together. Neelson (1880) using impure culture proposed a synthetic media containing ammonium tartrate, natural potassium phosphate, magnesium sulphate and calcium chloride for pigment production. Gessard (1892) found that phosphate ions were necessary constituents of above media. Thumm (1894) believed that in addition to organic sources of nitrogen and phosphates, $MgSO_4$ must be present for fluorescence to occur. Georgia & Poe (1931) definitely showed that Mg^{++}, $PO_4^{\equiv}$, $SO_4^{=}$, ions are necessary for fluorescin production.

Using *Ps. aeruginosa* Gessard (1890) showed that glycerol peptone agar provided an admirable medium for pyocyanine formation and Seleen and Stark (1943) found Gessard's glycerol peptone agar highly successful. Jordan (1899) reported that ammonium salts of succinic, lactic, acetic or citric acids could serve as adequate carbon and nitrogen sources for pyocyanine production, while Robinson (1932) also using *Ps. aeruginosa,* found no better media than Bacto peptone for producing pyocyanine. Burton et al. (1947) when studying amino-acid requirements for pyocyanine production found that more than one amino-acid is concerned with pigment production, alanine and leucine being equal or superior to casamino acids as the source of nitrogen in the production of pyocyanine.

A third pigment formed in Pseudomonad cultures which has received little attention in the past is the red pigment pyorubrin. This pigment is considered by Meader et al. (1925) to be as characteristic of B. pyocyaneus as pyocyanine itself. They found that each of 44 strains produced fluorescent pigment, of these three never produced a trace of pyocyanine, in spite of abundant pyorubrin production. Of the remaining 41 strains 36 produced pyocyanine in varying amounts, and of these all but three produced pyorubrin.

For many years the occurrence of green and brown stains has been noted in New Zealand wools, and have recently become the subject of investigation. It has been firmly established that they are caused by *Ps. aeruginosa* and the data presented here describes the pigment producing activities of the bacterium.

SEPARATION AND PURIFICATION OF PIGMENTS

The pigments produced by a *Ps. aeruginosa* strain isolated from a Corriedale fleece were separated by shaking a filtered culture solution with chloroform. The chloroform layer is blue and a green water soluble pigment is left in the aqueous layer.

Separation of chloroform soluble pigments and consequent purification of pyocyanine was achieved by taking pyocyanine up in 1% HCl as its red acid salt. The residual chloroform, contain-

ing other pigments, was then passed through an alumina column resulting in most cases in separation of yellow and pink pigments. The acidified pyocyanine after reconversion to alkaline form and extraction into chloroform was also passed through an alumina column to ensure further purification. Final purification of yellow pigment, hemi-pyocyanine, was achieved by sublimation in vacuo.

PIGMENT FORMATION IN CULTURES

All media used which produced pigment formed a green colouration at the surface of the liquid culture in 12-48 hours—at 25° C. At 37° C. pigment production was more rapid. If cultures are unagitated this surface colouration will remain, but on agitation or mixing due to temperature inversion, the colour will diffuse throughout the medium, oxidation causing the colour to increase in intensity.

The initial colouration is due to a green water soluble fluorescent pigment, which later is augmented by the chloroform soluble pigment pyocyanine. Pyocyanine is not found in its fully oxidised state in cultures since extraction from undisturbed cultures first gives a green pigment which is rapidly oxidised to blue when shaken in the air. At a lower level in the culture below the green surface layer the colour of the solution is yellow and exhibits a green fluorescence. Extraction of an aliquot from this level with chloroform initially leaves a yellowish-green fluorescent aqueous layer, and a clear chloroform layer. Subsequent oxidation causes the fluorescence of the aqueous layer to increase its colour and the chloroform layer develops a blue colour which is pyocyanine. This is also the case at the base of the culture, but the final quantity of pigment after oxidation is by no means as great (Table 1).

TABLE 1

Level of Culture	Pyocyanine—mg/25 ml. of culture medium
Surface Layer	1.20
Medial Layer	0.90
Basal Layer	0.15

Thus it is clear that in this case, pyocyanine is the result of oxidation of a leuco-base formed in the culture medium. Such oxidation was suggested by Young (1937), but the above results are not in accord with Turfitt (1937), who claimed that the strains used did not produce a leuco-base.

The proportion of the water soluble to chloroform soluble pigments varied considerably with the media employed, similar results were obtained by Turfitt 1937; Young 1937; Georgia & Poe 1931; Burton et al. 1948.

PYOCYANINE AND ASSOCIATED PIGMENTS

The chloroform extract from young cultures of 8-12 hours is non-fluorescent and contains nearly pure pyocyanine. The chloroform extract from the older cultures exhibits a marked blue-green fluorescence and upon purification is found to contain, as well as pyocyanine, several associated pigments all of which are soluble in chloroform and quite distinct from the green fluorescent pigment of the aqueous layer. After the first step in the purification of pyocyanine by taking up in 1% HCl as its red acid salt, the blue-green fluorescent pigment remains in the residual chloroform which is yellow by transmitted light. Subsequent passage of this chloroform fraction through an alumina column results in the deposition of at least three colours. These being, at the top of the column a pigment yellow by transmitted light and fluorescent yellow in ultra-violet, a pigment pink by transmitted light and non-fluorescent and also a very soluble bluish-brown pigment which has a brilliant blue fluorescence in ultra-violet light.

The yellow and pink pigments are both very soluble in dilute alkali and cannot be separated by eluting through the column with alkali. Separation can only be achieved by breaking the column and removing the alumina in sections. Analysis of these fractions clearly shows that these two pigments are not distinct pigments, but are oxidation-reduction products of the same pigment. This pigment is highly soluble in dilute alkali, giving a non-fluorescent wine coloured solution. It has low solubility in neutral and acidic aqueous solutions giving yellow and orange coloured green fluorescent solutions respectively. These properties plus its indicator reaction, point to it being hemi-pyocyanine (α-hydroxyphenazine). Melting point determinations and spectrographic analysis of the pigment at three pH levels show its great chemical similarity to pyocyanine. Hemi-pyocyanine is derived from pyocyanine by demethylation (Wrede & Strach 1928).

In view of the absence of hemi-pyocyanine in very young pyocyanine-producing cultures it has been concluded that its abundance in older cultures is due to the breakdown of pyocyanine, as also found by Schoental (1941).

EFFECTS OF AGEING ON PIGMENTS IN CULTURE AND IN PURE STATE

After 8-10 weeks the blue or greenish-blue colour of the culture turns to a dark red which when shaken with chloroform proves to contain only water soluble pigments. The chloroform soluble pigments originally present, that is blue pyocyanine and yellow hemi-pyocyanine, have all been converted to red water soluble pigments. The greenish-blue fluorescence remains unchanged.

When a pure non-fluorescent chloroform solution of pyocyanine is exposed to light and air, decomposition of the pigment occurs. The decomposition products have been shown to be predominantly hemi-pyocyanine. In aqueous solution similar degradation also occurs, the change being very slow at pH levels between 3.3 and 7.2, at pH 7.4 the pyocyanine is even more stable to photo-oxidation. At pH 3.3 when there is complete conversion of pyocyanine to its acid salt, no degradation occurs. However at pH 8.4 and above, pyocyanine is in its most unstable state and degrades to give a yellow solution of hemi-pyocyanine.

When solutions of the above pH values and with the same concentration of pure pyocyanine are first reduced by activated hydrogen the same reduction products as described by Friedheim (1931) are formed. When these solutions are removed from the reducing atmosphere and allowed to stand in air, re-oxidation back to the original colours tends to occur in all solutions. After 12 days of photo-oxidation little or no degradation of pyocyanine to hemi-pyocyanine occurs at pH 7.4 and below. At pH 8.4 and above, exposure for 3-4 days results in a colour change of pyocyanine from blue to pink. This pink pigment has been shown to be closely related to hemi-pyocyanine. Therefore the effect of reduction is to form a pink indicator at pH 8.4 after a period of oxidation; without reduction a yellow indicator is formed.

When initial pH of culture media is between pH 5-9 (values more or less than these will seriously retard or completely prevent growth of this bacterium), all media finally equilibrate at pH 8.4 after approximately 10 days of active growth. Pyocyanine concentration remains constant for as long as 42 days at this pH. But, as pyocyanine is continually acting in a respiratory capacity, being reduced by the bacteria and oxidised by the air (Friedheim 1931), it is believed that the final breakdown product of pure pyocyanine (pH 8.4) initially reduced prior to oxidation, is the same as that produced in culture at pH 8.4.

Neutralisation allowed the subsequent chloroform extraction from the aged, pink culture media of a very small quantity of hemi-pyocyanine. However in most cases extraction could not be achieved at all. In view of this it is believed that on ageing, the pink solution of hemi-pyocyanine becomes stable and loses its indicator properties.

SUMMARY

Four pigments have been isolated from a chromogenic strain of *Pseudomonas-aeruginosa* isolated from the fleece of Corriedale sheep. The pigments are:

(i) Green fluorescent water soluble pigment.
(ii) Blue fluorescent, chloroform and water soluble pigment.
(iii) Blue, chloroform and water soluble pyocyanine with indicator properties.
(iv) Yellow, chloroform and water soluble hemi-pyocyanine with indicator properties.

REFERENCES

Baerthleim, K. (1918), Zentr. Bakt. Parasitenk **81,** 1 428.

Breed, R. S., Murray, E. G. D., Hitchens, A. P. (1948), "Bergy's Manual of Determinative Bacteriology." 6th Edition.

Burton, M. O., Eagles, B. A., and Campbell, J. J. R. (1947). Can.J.Res. **C.25,** 121.

Burton, M. O., Campbell, J. J. R., and Eagles, B.A. (1948). Can.J.Res. **C.26,** 15.

Christie, R. (1948), Aust.J.Expt'l. Biol. & Med. Sci. **26,** 425.

Eisenburg, P. (1914), Zentr. Bakt. Parasintenk, i, **73,** 466.

Friedheim, E. (1931), J.Expt'l. Med., **54,** 207.

Friedheim, E., Michaelis L. (1931), J.Biol. Chem., **91,** 355.

Gaby, W. L. (1946), J.Bact., **51,** 217.

Gessard, C. (1890), Ann. inst. Pasteur, **4,** 88.

Gessard, C. (1892), Ann. inst. Pasteur, **6,** 801.

Gessard, C. (1919), C. R. Acad. Sci. Paris, **168,** 1066.

Georgia, F. R. & Poe, C. F. (1931), J.Bact., **22,** 349.

Jordan, E. O. (1899), J.Exp'l. Med., **4,** 627.

Lehmann, K. B., Neumann, R. O., Breed, R. S. (1931). Determinative Bacteriology. 7th German Edition, N.Y.

Meader, P. D., Robinson, G. H., Leonard, V. (1925), Am.J.Hyg., **5,** 682.

Neelson (1880), Cohn's Beitr. Biol. Pflinzen, 3 Heft II 187.

Robinson, G. L. (1932), Brit. Expt'l. Path. **13,** 310.

Sandiford, B. R. (1937), J.Path. Bact., 44, 567.

Schoental, R. (1947), Brit. J. Expt'l. Path., **22,** 137.

Seleen, W. A. & Stark, C. N. (1943), J. Bact., **46,** 491.

Thumm (1894), Arb. Bakt. Inst. Karlsruhe, **1,** 290.

Turfitt, G. E. (1937), Biochem. J., **31,** 212.

Wrede & Strach (1928), Z. Physiol. Chem., **215,** 67.

Young (1947), J. Bact. **54,** 109.

THE UNCONTROLLED USE OF THE ANTIBIOTICS

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In most hospital laboratories throughout the world, the increasing lack of sensitivity of micro-organisms to the antibiotics has been noted. In this article a summary of only a few hundred cases of infection with *Staphylococcus aureus,* with reference to the relation of this infection to treatment with various antibiotics will be discussed.

The ambiguous wording of the title of this article is as confusing as have been the mixed methods of control of the antibiotics. The various "Powers that be" and the Hospital Bacteriologist have all striven to curb the unnecessary use of antibiotics, but even today the expression is heard "Oh, I always use .....mycin," perhaps a most cherished and valuable antibiotic for the control of certain fatal infections. It is hoped that in the following lines it will be shown that the continued extravagant use of antibiotics, with or without laboratory control, will destroy their effectiveness.

The first case in this laboratory in which a general antibiotic insensitivity was found to be of serious clinical import was one in which *S. aureus* was found in the pus developing from an orchidectomy and epididectomy wound of Mr D.S. This organism, cultured on 28/9/54, was found to be insensitive to penicillin, streptomycin and aureomycin, but sensitive to chloromycetin. The patient had been treated for his tuberculous condition in this hospital with streptomycin—he had also had penicillin and terramycin—but was later transferred to the Sanatorium. On 14/2/55 the organism was found in his thoracotomy wound, but he eventually recovered from the infection.

Five months later, in April, 1955, three more patients, Mr M., Mr S. and Mr E., were discharging "insensitive" staphylococci from their thoracotomy wounds. Mr E. died of staphylococcal pneumonia. From this time onwards a number of minor infections with insensitive *S. aureus* were noted in Canterbury.

An epidemic of staphylococcal infection in a private maternity hospital in Christchurch culminated in November, 1955, with the death of eight babies from staphylococcal pneumonia. Several mothers had breast abscesses and cultures from these and from post-mortem material from the babies revealed *S. aureus* of the same phage type, namely 42E/7/73. Strains isolated from a variety of patients, ex-patients and nurses of the private hospital were of the same or related phage type.[i] Typing was done at the National Health Institute.

At about this time the infection was manifesting itself in this hospital, and cultures from patients' wounds and from housemen (53%), visiting and nursing staff (52% in the children's ward) very frequently showed the same phage type. These persons were placed in semi-isolation. Nasal swabs from laboratory staff, those visiting and those not visiting the wards, showed only one out of twenty to have insensitive *S. aureus*.

The table below sets out the *in vitro* sensitivity of *S. aureus* isolated from purulent discharges and from a few nasal swabs for *every third month* for the three years ending 30/11/55.

PERCENTAGE SENSITIVITY OF *S. AUREUS* IN PURULENT EXUDATES OVER A 3-YEAR PERIOD

	Penicillin			Streptomycin			Aureomycin		
	1953	1954	1955	1953	1954	1955	1953	1954	1955
% Sensitive	50	40	45	80	68	70	90	79	72
% Insensitive	35	38	40	16	24	23	7	12	21
% Moderately Sens.	15	22	15	4	8	7	3	9	7
Number of Tests	468	460	802	463	465	801	387	415	780
Increase in Resistance	1/7th times			½ times			3 times		

	Terramycin			Chloromycetin			Erythromycin		
	1953	1954	1955	1953	1954	1955	1953	1954	1955
% Sensitive	—	—	73	96	90	74	—	—	89
% Insensitive	—	—	24	2	6	31	—	—	7
% Moderately Sens.	—	—	3	4	4	5	—	—	4
Number of Tests	—	—	753	415	440	238	—	—	209
Increase in Resistance	1 in 4 now insens.			15 times			1 in 14 now insens.		

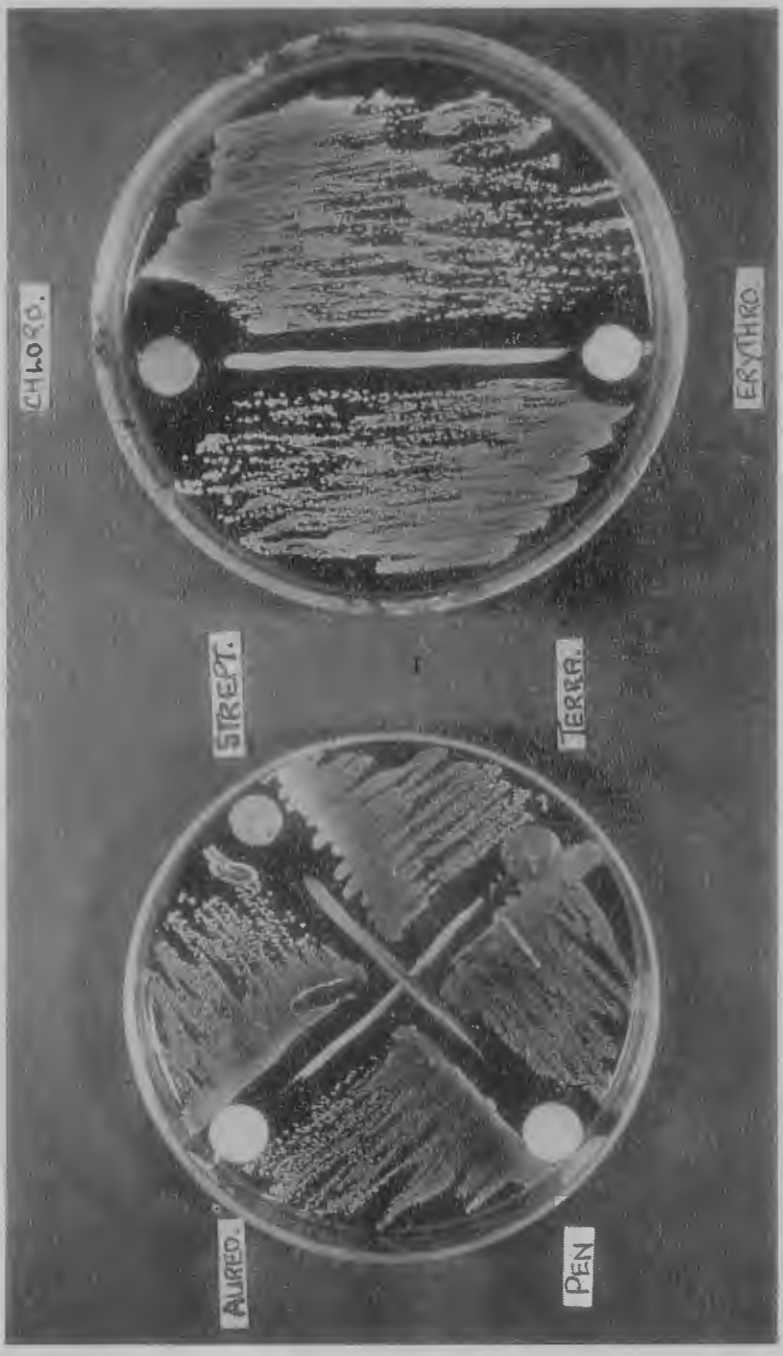
Legend: Sensitive = A radius of inhibition equal to that of Oxford H. Staphylococcus (about 2 cms.).
Insensitive = No inhibition, with the control showing about 2 cms.
Moderately Sens. = A radius of inhibition equal to half that of Oxford H. Staphylococcus.
— = Test not done.

Media used was 5% human blood agar.

Sensitivity was determined by the impregnated disc method using those prepared in the laboratory, each containing approximately 20 units of penicillin and the remainder approximately 150 micrograms of each antibiotic.

A control stroke of Oxford H. Staphylococcus, showing an inhibition of approximately 2 cms. was put up with all cultures.

A study of the table above reveals the following points:


CHLORO.
STREPT.
TERRA.
AUREO.
PEN
ERYTHRO

(a) There has been an increase in the number of cultures of *S. aureus* showing insensitivity *in vitro* to penicillin, streptomycin and aureomycin.

(b) After only about one year's bacteriologically controlled use of terramycin, chloromycetin and erythromycin, there is a fairly high proportion of insensitive cultures from infected wounds.

(c) Though the number of cultures insensitive to penicillin is greater than those insensitive to the other five antibiotics, this resistance has not appreciably increased over the last three years.

(d) Almost twice the number of cultures have been carried out in the third year as were done in the first two years. This has slightly weighted the figures.

(e) The wider use of antibiotics progressively reduces their effectiveness.

(f) The table shows a remarkable similarity to that given in an article on this subject reported from Australia [ii] although the number of tests recorded there are smaller.

In conclusion may be mentioned the ever-present risk of inducing staphylococcal enteritis by the administration of certain antibiotics. It is to be hoped that this is only a passing phase in the history of what should be the treasured antibiotics and that, with greater care in their use, this grave danger may pass. When this condition is suspected, it is very definitely the role of the laboratory to recognise it with the *utmost celerity*. A stained film of faeces, showing few other organisms than those resembling staphylococci may clinch the diagnosis and save the patient's life by the *immediate* administration of another suitable antibiotic. A heavy, or sometimes a pure growth of *S. aureus* which is insensitive to the earlier known antibiotics, will confirm the stained film findings the following morning.

The plate shows photographs of a pure growth of *S. aureus* in the primary cultures of faeces from a case treated with achromycin.

CONCLUSION

May the hand of the antibiotic administrator be stayed by necessity, judgment and by *in vitro* tests carried out with speed and accuracy.

SUMMARY

Excessive and uncontrolled use of the antibiotics, as well as failure to make use of available laboratory facilities to test their effectiveness has been shown to have reduced their value as therapeutic agents. The most common infecting organism, *Staphylococcus aureus,* has, after a few years' respite, again become a potential

menace to surgery. Fatal staphylococcal enteritis may follow antibiotic therapy given for some other illness.

ACKNOWLEDGEMENT

I wish to thank Miss Pamela Shearer of this department for the large amount of work which she did in searching the records to provide data for most of the figures given in this article.

REFERENCES

(i) Burston, J. & Barber, M.: "Antibiotic-resistant Staphylococcal Infection . . ." Lancet, 2: 578-582, Sept. 17th, 1955.

(ii) Edelman, L. V.: "Observations on the Sensitivity of some B. Coli and Staph. Aureus to some Antibiotics." Australas. J. Med. Tech., 1: 75, July, 1955.

(iii) Suie, T.: "Insensitivity to commonly used Antibiotics . . ." Am. J. Ophth., 40: 425, Sept., 1955.

SALMONELLA REFRESHER COURSE

This course conducted by Mr S. W. Josland was held at the National Health Institute from November 7th to November 25th, 1955, and was attended by twelve bacteriologists.

The practical work was carried out in the teaching laboratory of the Institute which is admirably equipped and stocked with media for such courses. Each morning the group met in the Library to read the general bacteriology of the Salmonella Group and also to discuss any problems or points of interest arising out of the previous day's practical work.

The following is a brief description of the work carried out during the course.

Each worker was supplied with the following sera:

"O" sera (absorbed Burroughs Wellcome)	"H" sera (Prepared by S.W.J.)
Polyvalent O (A-E)	P.S.A.
Factor II	P.S.B.
IV, V	P.S.C.
VI, VII	P.S.D.
VIII	Factors a, b, c, d
IX	eh, enx, fg, gm
Vi	mt, i, k, lv
III(X, XV, XIX)	p, r, y, z
XI	
XIII, XXII	

Cultures for the above somatic groups, and formalinised antigens for each of the flagellar sera were provided.

Each of the "O" sera were then tested by slide agglutination against each of the known cultures and the results recorded. This exercise demonstrated the usefulness of absorbed somatic sera and also showed if the sera as obtained from the makers were completely absorbed or not.

Each of the "H" sera except P.S.A., P.S.B., P.S.C. and P.S.D. was titred against its homologous antigen using the formalinised antigens provided.

Single factor X, XV, XIX, f, t and v sera were needed at times during the course and these were successfully prepared during an exercise in agglutinin absorption.

An exercise in phase variation was done on a culture of S. typhi-murium. This culture was first tested quantitatively for the presence of phase I and phase II antigens (i - 1, 2). Then two tubes of semi-solid agar were melted and cooled to 50° C. To one tube was added four drops of i serum and to the other, four drops

of 1, 2 serum. The plates were poured and then inoculated. After incubation overnight a subculture from the edge of each plate was made into Tryptose Phosphate Broth and incubated 5-6 hours. Each broth culture was then tested quantitatively for the presence of both i and 1, 2 antigens. During this exercise it was shown that it is sometimes necessary to pass the organism through two or even three semi-solid plates containing serum in order to drive the organism over into the required phase.

At the conclusion of these exercises we were given cultures of the eighteen salmonella types so far isolated in New Zealand.

These salmonella types were then serologically identified and full notes made on the methods used.

SALMONELLA TYPES ISOLATED IN NEW ZEALAND

S. paratyphi A	S. pullorum
S. paratyphi B	S. typhi
S. typhi-murium	S. enteritidis
S. arechavaleta	S. dublin
S. derby	S. anatum
S. saint-paul	S. orion
S. bareilly	S. weltevreden
S. cholerae-suis	S. newington
S. bovis morbificans	S. senftenberg

Some thirty-two other salmonella organisms along with Sh. sonnei, Proteus mirabilis, E. coli 055 and a Paracolon were presented to us to work out their antigenic formula.

Each pure culture was treated in the following manner.

1. Test the organism against Proflavine to determine whether the culture is smooth or rough.
2. Test the organism against the panel of "O" sera.
3. Subculture into semi-solid agar. This demonstrates motility or non-motility.
4. Subculture from edge of growth in the semi-solid plate into Tryptose Phosphate Broth which is incubated at 37° C for 5-6 hours.
5. Formalinise the broth culture and test against the polyvalent "H" sera P.S.A., P.S.B., P.S.C. and P.S.D. If agglutination occurs with any of these four sera test further with individual sera contained in the polyvalent serum.

With the results obtained from these tests and reference to the Kauffman White Schema the identity of an organism can be worked out.

During the last week of the course three lectures were given.

"Salmonella Infections in Animals and Birds in N.Z.," by Mr R. M. Salisbury of the Wallaceville Animal Research Station.

"Phage Typing" by Dr. J. D. Manning of the National Health Institute.

"Public Health Aspects of Salmonella Infections," by Dr. F. S. Maclean, the Director of the Public Hygiene Division, Department of Health.

From this brief description of the course it can be seen that we were able to gain confidence and a fuller understanding in the use of sera for the identification of salmonella organisms. We also know how far we can go in a hospital laboratory with the identification of the more uncommon salmonella types met with in N.Z.

Mr Josland, Dr. Manning and members of the staff from the Institute had a vast amount of additional work to do in conducting this course which was fully appreciated by all those attending.

G.R.R.

COLORIMETRIC CHEMICAL PATHOLOGICAL ESTIMATIONS USING THE LOVIBOND COMPARATOR

Edited by: G. J. Chamberlin.

Published by: The Tintometer Ltd., Salisbury, England. pp. 116. Price 18/6.

Described as a handbook of methods and information. The book is well set out. 32 separate determinations are described and the publishers promise further additions and invite enquiries and suggestions for new tests. As well as describing the actual technique of the tests an interesting, concise and useful introduction is given to each type of estimation, giving reasons for requiring the test and information to be gained from the results obtained.

Many routine hospital laboratory estimations on plasma and blood such as bilirubin, cholesterol, haemoglobin, Lange's colloidal gold reaction, phosphatase, proteins and sugar are covered, as well as those less frequently encountered such as bromide in blood, D.N.O.C. (Dinitro-ortho-cresol) in blood, mepacrine in plasma and urine, phenylpyruvic acid in urine, P.A.S. (p-amino-salicylic acid) in blood.

References are given for further information or elaboration from original works. Several pages are devoted to notes on colour measurement.

The Lovibond comparator has seen many years' service as a laboratory tool and because the reviewer of this handbook has had limited experience with it he is not in a position to evaluate it and will confine himself to a description of the handbook alone.

The comparator is available in two models—a pocket size and an all-purpose model. It consists of a moulded plastic case with an opal glass screen at the back and two compartments to receive test tubes or rectangular cells containing the liquid under examination. In the front portion are two circular holes situated side by side opposite to the opal screen and coinciding with the vessels under examination. A moulded disc fitted with nine glass colour standards fits into a recess in the lid of the comparator and can be rotated in front of the cell compartment.

Discs are available for numerous quantitative chemical and clinical tests. Most of the tests for which the comparator is used depend upon (a) the change in colour which takes place, or (b) the intensity of the colour developed, when a measured quantity of an indicator solution or reagent is added to a measured quantity of the test sample. The initial colour of the test sample naturally affects the colour developed, so provision is made for a "blank" or

untreated sample of the test solution to be placed behind the colour stand when necessary, in a vessel of the same internal diameter as that containing the test sample to which the indicator or reagent has been added. This arrangement automatically compensates the colour standard for any inherent colour in the solution under test and thus ensures that the same colour standards are applicable for use with either coloured, slightly turbid, or water-clear solutions.

Discs containing sets of arbitrary colour standards for use in the grading of certain natural products are available. Also, special discs may be prepared in which the colour standards are to match a series of coloured samples supplied by an individual customer.

The glass standards are carefully prepared and critically checked in pathology laboratories and research institutes and are fadeless and permanent.

Accuracy will depend on care in handling and purity of reagents as in all laboratory procedures. No elaborate claims seem to be made for accuracy.

Where day to day comparisons of levels rather than absolute values are required, the system seems to meet a real need in a busy laboratory.

The stipulation that many biochemists make that the standard and test be subjected to the same conditions and treatment is well known and undoubtedly sound where highest accuracy and top quality work is required. Whether this may be overlooked and whether ready-prepared standards are satisfactory is over to the individual to decide according to his needs.

Particularly in a small laboratory this equipment could be valuable as the outlay is far less than for any other apparatus giving such a wide range of service.

F.L.N.C.

SUMMARY OF REPLIES TO QUESTIONNAIRE REGARDING EMERGENCY CALL DUTY

1. Has the emergency call increased during the last two years?
   81% of the laboratories report that the volume of work has increased during the last two years.
2. What are your weekly hours of work?
   In most instances hours quoted have been seven hours per day and three to three and a half hours on Saturdays, totalling thirty-eight to thirty-eight and a half hours per week. Some laboratories (33%) detail a forty-hour week either by taking a thirty-five minute lunch hour or working 8.30 a.m. to 5.30 p.m., and in these cases Saturday morning is counted as overtime.
   We feel, however, from our own experiences and observations in other laboratories, that tools are not "downed" at 5 p.m.
3. What proportion of the staff are on regular emergency call duty?
   The proportion of staff depends on the ratio of Juniors: Seniors.
4. At what stage of training are your staff eligible for emergency call duty?
   In laboratories where there is more than one person available to take call duty the standard is either at intermediate level or when the senior is satisfied with the capabilities of the staff on call.
5. Is the emergency call duty extra to normal working hours?
   94% of the laboratories reported that the emergency call duty was extra to normal working hours.
6. If so, is it compensated by (a) Free time, (b) Financial reward, (c) No compensation.
   (i) *Where forty hours per week are worked:* Unqualified or ungraded staff receive financial reward. Graded officers are allowed time off. 15% of laboratories report that all the staff receive financial reward for emergency call duty. 15% of laboratories report that there is no compensation. (ii) *Where less than forty hours per week are worked:* Free time is given to qualified staff. Juniors or unqualified staff receive financial reward.
7. When required to be on emergency call duty are you available (a) at home, (b) at the department, (c) resident in the department?
   In all instances the staff on call are available at home or at a point of nomination.

8. Does the emergency call duty involve routine work as well as urgent work?
   15% of laboratories report that emergency call duty involves some routine work.
9. Who is available for urgent work from 11 p.m. to 8 a.m.?
   With the exception of two of the main centres where pathologists are on call from 11 p.m. to 8 a.m. the duty is taken by the staff responsible.
10. What type of work is mostly dealt with during these hours?
    There is a similarity in the type of urgent work requested—e.g.,Cross-types, Coomb's testing, Haemoglobins, W.B.C. and differential counts, Blood Sugars, Diastases, T.N.P.N., Swabs, etc., for sensitivities and C.S.F.'s.

SUMMARY

We are at a loss to understand why our request for information on a pertinent subject met with no response from several laboratories—namely, Auckland Central, Middlemore, Dannevirke, Timaru and Waipukurau, but we appreciate the co-operation of those who replied so readily.

The volume of emergency call duty shows a marked increase.

A disquieting feature is that 15% of laboratories are involved in routine work during emergency call hours. It is evident that where a pathologist is called upon to screen "urgent" work that its volume decreases markedly.

In sole charge laboratories it is obvious that the bacteriologist is on constant emergency call duty and is therefore forfeiting the full free time which he might reasonably expect. Where staffs are larger and proportions of up to 50% are available for call duty. people qualifying for free time find difficulty in availing themselves of it.

Is this because laboratories are inadequately staffed?

The tenor of these replies does not appear to be consistent with the opinions expressed at the July, 1955, conference, so, do the answers to the questionnaire show the situation as it really is or as it is supposed to be?

STAFF COMMITTEE,
Pathology Department,
Christchurch Public Hospital.

JUNIOR ESSAY COMPETITION

Entries for both the Technical Study and for the Essay Sections of this competition close with the Hon. Secretary on June 30, 1956. Entrants must state for which section they wish to enter and, on a separate sheet of paper, give their name and address. A prize of £2/2/- is offered for the best entry in each section if it is of a reasonable standard.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

Christchurch Hospital

THEORETICAL EXAMINATION: Wednesday, 19th October, 1955, at 9.30 a.m.

Time Allowed: 3 hours.

All questions should be attempted.

Examiners: Dr. F. Gunz, Mr D. H. Adamson

1. Describe in detail your method of examining a pleural fluid for Mycobacterium tuberculosis. Enumerate the constituents of the reagents used. (15 marks)
2. Give the technique of determining the total and differential leucocyte count in a sample of oxalated venous blood. To what errors are these methods subject, and how may they be minimised? (15 marks)
3. You have received a specimen of cerebro-spinal fluid. State your method of estimating its content of:
   (a) Sugar, (b) Chloride, (c) Protein, giving the normal values in each case. (20 marks)
4. Describe in detail how you would determine the packed cell volume of a sample of blood. Define the terms "Mean Corpuscular Volume" and "Mean Corpuscular Haemoglobin Concentration." How are these values calculated? (15 marks)
5. Describe precisely the method used in your laboratory for the isolation of N. gonorrhoeae from a urethral discharge. State what you know of the morphological, cultural and biochemical characteristics of this organism. (15 marks)
6. Write a brief essay on the prevention of personal accidents in the laboratory. (20 marks)

PRACTICAL EXAMINATION—PART I—Wednesday, 19th October, 1955, at 2 p.m.

Time Allowed: 3 hours.

All questions should be attempted.

1. (a) Determine the percentage of urea in the urine "A". Indicate how you arrive at the figure from the reading. (1.3 - 1.4%). (35 marks)
   (b) Name the three pipettes, "B", "C", & "D" and describe briefly how and for what purpose each is used. (Certified transfer, 1 ml. Ostwald-Folin, 0.2 ml. Micro-Grad. 0.001). (15 marks)
2. (a) Perform a reticulocyte count on the stained film "E." (4-6%). (15 marks)
   (b) Examine and report upon the three stained blood films "F", "G", & "H." (Inf. Mononucleosis, Erythroblastosis, Chronic myelogenous leukaemia). (15 marks)

(c) Determine the ABO group of the blood "I" by the tube method. Tomorrow morning you will be provided with a choice of donor bloods and required to cross match specimen "I" with the donor's blood you consider suitable. (Group AB).

(20 marks)

3. The swab "J" is charged with the bloodstained discharge from a child's nose. Report upon direct films. Inoculate suitable media and test one of the cultures for sensitivity to four antibiotics, using the discs on your bench. (Cultures will be examined tomorrow morning).
(Pus scanty, staph. numerous, strep. moderate, diptheroids numerous.)
(40 marks)

4. (a) Examine and report upon a wet film made from the urine deposit "K".
(Pus. mod. R.B.C.'s numerous. Triple phosphate crystals scanty).
(Orgs. profuse, also amorphous phosphates mod., Yeasts scanty).
(10 marks)

(b) Find the titre of Brucella abortus antibodies in the serum "L" (Titre is below 2000).
(480-960 (800))

(20 marks)

5. (a) Tie the cork into the bottle "M" containing C.S.F. Pack the specimen in the box provided, wrap it and address it to the pathologist at the Wellington Hospital so as to reach him as speedily as possible.

(5 marks)

(b) Make a bacteriological loop with the platinum wire provided.
(5 marks)

(c) Draw two Pasteur pipettes from a length cut off the quill tubing provided.

(5 marks)

6. (a) What is article "N"? Is there anything defective about it? How does it function? (Thermostat capsule overheated and exploded).
(5 marks)

(b) What are the articles "O"? Why is one more efficient for certain purposes than the other? (Liebig condensers—coiled, quicker for ether, etc.; uncoiled—for general purposes.)

(5 marks)

(c) What is the article "P"? For what is it used? (Fuchs-Rosenthal counting chamber—C.S.F. and eosinophil counts).

(5 marks)

PRACTICAL EXAMINATION—PART II—Thursday, 20th October, 1955, 9.30 a.m.

Followed by Oral Examination.

1. Yesterday you grouped the specimen of blood "I". Select a suitable donor from the 3 specimens provided and do a tube cross match. (Provided A. B. and O., select A. or B.)

2. Examine and report on the cultures from swab "J". State what further investigations would be required to identify the predominant organism. (C. diphtheriae—Glucose, Sucrose, Dextrose, Tellurite, Virulence).

ORAL EXAMINATION—11 a.m.

Subjects discussed at the Oral Examination, which was completed within about an hour were:

Isolation of the Salmonellae from faeces, isolation of Brucella abortus from the blood, use of different oxalates for blood for haematology and for biochemistry, the difference between plasma and serum, reason for the "battlement" method for differential counts, haematocrit technique, blood grouping, haemoglobinometry, appearances of individual blood cell precursors, bacterial nomenclature.

At a meeting held after the announcement of the examination results both examiners discussed with the candidates in general terms the main shortcomings which they had noted in the papers and practical work, and made suggestions which should help those sitting the Final Examinations to present their knowledge in a form more concise and acceptable to their future examiners.

THE EXAMINERS' COMMENTS:

The fact that all candidates passed does not mean that we were entirely happy with some of the performances. In general, candidates displayed a good deal of carelessness, bad spelling and bad arrangement in the Theoretical Examination, and the essay on "The Prevention of Personal Accidents in the Laboratory" was particularly badly done. We think that this is an important subject which should be more stressed during training. The Practical Examination was on the whole better done than the Theoretical, but we feel that considerably more attention to points in technique, particularly in blood grouping, is necessary. The Oral Examination frequently showed a lack of understanding of the rationale behind certain tests and techniques. The bacteriological part was mostly quite successfully done, and practical work was reasonably satisfactory.

THE CANDIDATES' COMMENTS:

Perhaps the written paper was rather too long and half an hour too short a time to set out an essay. The practical work seemed a little much to carry out successfully in unfamiliar surroundings. The general arrangements and the apparatus supplied for the examination were very satisfactory and the questions were fair.

CANDIDATES WHO PASSED WERE:

Mr W. Aldridge, Wellington Public.
Miss A. Dunnachie, Lower Hutt.
Miss D. Hitchcock, Wellington.
Miss M. Kynoch, Waipukurau.
Mr J. Lyon, Wanganui.
Miss J. Maitland, Christchurch Public.
Mr R. Smail, Kew, Invercargill.
Mr B. Smith, Timaru.

All persons who presented themselves for this examination were successful.