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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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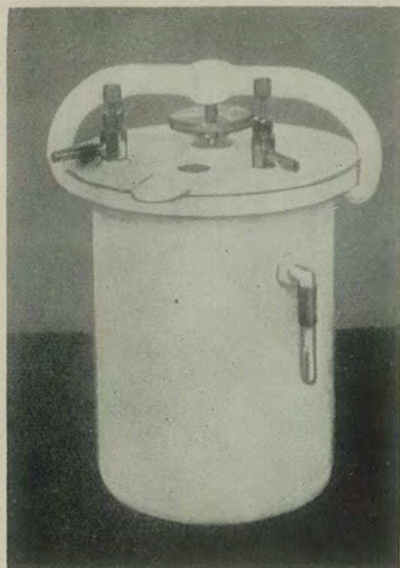

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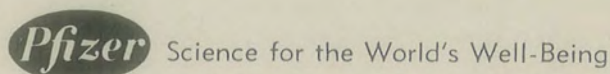

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STAPHYLOCOCCUS TOXINS AND THE COMMERCIAL PRODUCTION OF TOXOID

R. T. D. AITKEN

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HISTORICAL REVIEW

The evergreen Staphylococcus has provided and will continue to provide stimulus and material for many papers, academic and technical, in the fields of Bacteriology and Immunology. It was of interest to note that in a recent book on the Staphylococcus, the bibliography contained no fewer than 4,000 references on the subject. (Elek (1959).)

My discourse will add nothing to the sum of theoretical knowledge of the subject but will review the accumulating knowledge of the products of the Staphylococcus with special reference to the alpha toxin and show how this knowledge is applied in the manufacture of a biological product

Interest in the filterable products occurring in staphylococcal broths was evident as early as 1894 when Van de Velde recorded his observations on the leucocidal activities of the Staphylococcus. Kraus and Clairmont (1900) and Neisser and Wechsberg (1901) demonstrated active haemolysins. The latter showed the diffusible nature of the haemolysins, identified them as antigens producing specific antibodies, and so recognised their exotoxic character. They also confirmed the work of Van de Velde on leucocidins. Kraus and Pribam (1906) showed that broth filtrates were lethal to rabbits in thirty minutes and demonstrated that haemolysin could be neutralised by antibody. It is worthy of note that today, standardisation of staphylococcal toxin and antitoxin is based on neutralisation procedures. Though Nicolle and Cesari (1914) confirmed this work and recorded the dermonecrotic effect of small doses of toxin, it was to have little recognition in the interpretation of the nature of staphylococcal infection in man. Ten years passed during which the pathogenicity of the Staphylococcus was assigned to endo-toxins and host resistance was believed to devolve on opsonins and phagocytosis. These were the principles of the Almroth Wright school of thought and were the theoretical basis of vaccine therapy. The success of vaccines was thought to confirm the opsonin theory which was to be accepted for many years, and which to my knowledge as not been disproved. It is, however, but a small part of the denfensive mechanism of the host.

Stimulated no doubt by the Dick's work on scarlet fever toxins (1924a, 1924b), Parker (1924) prepared broth cultures in

an atmosphere of 10% CO_2 and was able to produce higher titres of staphylococcal toxin than had hitherto been obtained. She confirmed earlier work on the haemolytic and dermonecrotic effect, and the lethal characteristics of the filtrate on rabbits. Bigger, Boland and O'Meara (1927) showed that staphylococcal cultures on agar produced more toxin than in broth cultures, and they inadvertently killed five out of six rabbits when attempting to produce antitoxic serum. Bigger (1937) later expressed the hypothesis that successes with staphyoloccal vaccines prepared in the usual way were due not to opsonins but to toxin washed from the agar cultures along with the bacterial cells.

The next milestone in knowledge of the Staphylococcus and its products occurred in 1928 (Kellaway et al., 1928). This unfortunately was a medical disaster and involved the deaths of twelve children who had been inoculated with a diptheria toxin-antitoxin mixture from a 10 ml. multidose phial that had been accidentally contaminated with a pathogenic Staphylococcus. The organism had multiplied in the phial of antigen which for particular reasons had been issued without a bacteriostatic substance. The toxicity of the contaminated preparation was such that doses of the order of 0.2 ml. proved fatal to twelve of twenty-one children. Post mortem results suggested that the deaths were toxaemic in nature, and at the time it was thought to be due to *in vivo* production of toxin by the injected viable staphylococci. This was the Bundaberg disaster which was to have far reaching effects. Burnet (1929, 1930) carried out further research on the nature of staphylococcal toxin, confirming the work of preceding years, and succeeding in producing higher titres of toxin than had previously been recorded. The most prolific and rapid toxin production was achieved in sloppy agar gels under an atmosphere of 20% CO_2. Toxin was obtained by mincing the gel and expressing the fluid through filter paper. Commercial toxin production was to be based on Burnet's work during the 1930's. Burnet (1929) was the first to show that staphylo-toxin, like diptheria and tetanus toxins, could be toxoided by the addition of small amounts of formaldehyde, that is, toxicity could be annulled, but antigenicity kept intact.

Bundaberg had given clinical significance to earlier laboratory findings and the staphylococcus was accepted as a toxigenic pathogen.

During the 1930's interest in staphylococcal toxins led to considerable work on the subject. From preceding work it was evident that the haemolytic characteristics of the toxin were a useful index of toxin production. Various workers, however, began to record

conflicting results from their observations, and former simple hypotheses became untenable. The use of erythrocytes from various species while leading to confusion in the first instance, was the means by which the complex nature of the toxin was elucidated. Bigger (1933) had also noted a peculiarity in haemolysin tests, the phenomenon of "hot-cold lysins". Tests carried out at 37°C would proceed to much higher titres if placed in the ice box overnight. Glenny and Stevens (1935) were to resolve the immediate problems by identifying α— and β— lysins, the former haemolytic to rabbit and sheep cells, the latter only to sheep cells. Bigger was, in fact, observing a characteristic of the β—lysin.

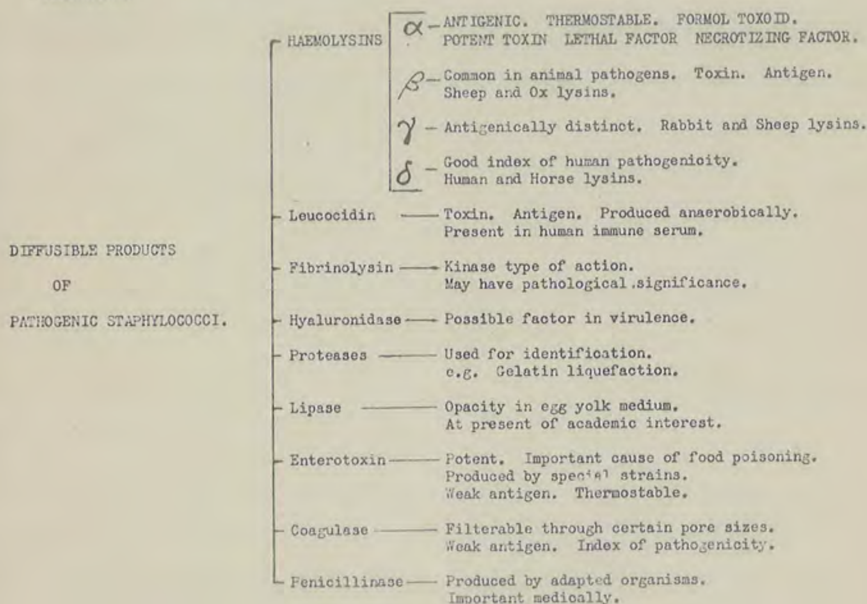
Leucocidin, the third known staphylococcal toxin at this time, was also causing confusion—some identified it with α— toxin while others correctly assumed it to be a distinct entity. Valentine (1936) clarified this issue by showing that α— toxin did destroy rabbit leucocytes but not human leucocytes. Certain strains of staphylococci, however, produced a factor lethal to rabbit and human leuococytes but had no action on red cells. A year later Proom (1937) showed that conditions for maximal yields of α— toxin and leucocidin differed.

Leucocidin is produced under anaerobic conditions, is a true toxin and it is antigenically distinct from α— and β— toxins. At this time McClean (1937), reported the interesting finding that the function of agar in producing high yields of toxin was one of adsorption from broth substrates, substances inhibitory to toxin production. Agar was replaced in his experiments by kaolin, Keiselguhr, cellophane and filter paper and equivalent toxin yields were obtained. Casman (1938) omitted agar from his broths and bubbled a carbon dioxide-oxygen mixture into his cultures. A step such as this may have considerable commercial significance as it is usually the first approach to deep fermentation. Further work by Duthie and Wylie (1945) showed that high titre of toxin could be rapidly produced with adapted strains by the mechanical rocking of cultures which were supplied with an atmosphere of 20% CO_2 and 80% O_2. This brings up to date knowledge of the technique of staphylococcal toxin production, and methods of commercial production are usually based on one of these later techniques.

The discovery and subsequent increase in knowledge of other diffusible products of the staphylococcus ran parallel to, if at a lesser tempo than the development of knowledge of the properties and preparation of the α—toxin. Most notable amongst these were the enterotoxin first recognised by the American bacteriologist Jordan (1930) and the staphylococcal coagulase first described by

Loeb as early as 1903. Fibrinolysin production by staphylococci was demonstrated by Minett (1936) and hyaluronidase by Schwabacher et al. in 1945.

The diagram summaries the present known diffusable products of the staphylococcus and it will be noted that γ—and —δ lysins have been postulated to account for the observations of later workers.



TOXOID PRODUCTION

STRAINS:

As there is a high correlation between α— toxin production and pathogenicity, most pathogenic strains will produce toxin to a moderately high titre in appropriate medium and when optimum condition of growth obtain. In commercial production, however, it is the practice to use known strains that have previously been used for such a purpose. Two important characteristics are expected of such strains, the first is their toxin producing capacity, and the second their capacity to remain prolific toxin producers; that is, they do not readily undergo dissociation related to a disadvantage in toxin production. These strains which I regard as biological freaks are known as toxicogenic mutants. These mutants occurring by chance in laboratory flasks or industrial fermenters are selected on account of their high toxin producing capacity and occasionally "run true" with regard to their acquired

characteristics. These become classic strains and are of great value in commercial production. S. aureus, Wood 46, is such a strain and has been widely used commercially. In appearance Wood 46 gives no hint of its usefulness. On human blood agar it looks like an old laboratory culture with small colonies, scanty pigment production, and weak haemolysis. Burnet, commenting on his most florid toxin producers, noted that they were usually albus variants.

In commercial practice standardisation of technique has a special position. A standard technique with a good safety and potency record assumes more and more importance on each successive run. The given technique ultimately achieves "built-in" factors of safety and potency and the selected organism becomes part of the system. A manufacturer therefore tends to be conservative in his selection of organisms for production runs.

However, I shall record that S. aureus phage type 80/81 has achieved a haemolytic titre of 1/1000 under ideal conditions of growth and in the unadapted state. This would be classed as a moderately high yield of α— toxin.

MEDIUM

Tryptic digest ox heart broths are used for toxin production. Experiments to date with chemically defined, semi-synthetic media based on casein hydrolysate have resulted in low yields of toxin. Frequently, however, the pattern in development is towards the chemically defined medium. It is able to be accurately reproduced from batch to batch, is usually cheaper to produce, and when purification of the final product is the object, methods for the elimination of residual medium are more readily designed. Also, the final product, if a culture filtrate, is at an advantage compared with digest substrates with respect to physiologically reactive nitrogen fractions of polypeptide and peptone nature.

The production of α— toxin on semi-synthetic media in practice may be difficult and may depend ultimately on the occurrence of a suitable mutant. Requirements for toxin production are usually much more exacting than requirements for growth.

It is my practice to avoid media on commercial peptone for toxin and vaccine production. My reason for this is the assumption that bacterial decomposition would be difficult to control in proteins used in large scale peptone production. Spore-bearing organisms may also be observed in large numbers in some peptone powders, and histamine-like substances have also been noted in peptones.

In contrast, tryptic digests prepared with fresh meat can be kept free from non-specific bacterial products and the metallic ion content of the medium can be controlled when this is necessary.

PLANT

Commercial production may make use of multiple units such as the Roux bottle or Fernbach flask, though substitutes for these, such as square gin bottles or milk bottles are permissible if soda glass is without effect on the substance being prepared. When increased volumes are required and the inoculation and harvesting of large numbers of small units becomes impractical and uneconomical large, flat, glazed, earthenware vessels containing a litre of medium in a shallow layer may be used. Vessels able to be stacked take precedence for volume production and incubator rooms replace the familiar box type incubator.

The manufacturer will always replace unit production methods with deep fermentation where it is possible. The transition, however, may be extremely difficult and again specially adapted strains may be necessary for success. A considerable amount of laboratory and pilot scale work precedes such a change-over in production methods.

My early experiments on staphylococcus toxin production were carried out in a large pressure cooker with an epoxy resin lining. Agitation was by a rotating centrally-positioned stainless steel impellor and the surface of the medium was blown with sterile air. Temperature was controlled by partially immersing the vessel in an agitated, thermostatically controlled 37°C water both. Though a heavy growth of staphylococcus resulted, toxin production was nil.

Production is at present carried out in 10 litre spherical flasks containing 5 litres of broth which are rocked slowly on a specially constructed platform. Stainless steel tubes are arranged to permit the ingress and egress of 20% CO_2-80% O_2 mixture, and to allow inoculation and later withdrawal of samples. The gas mixture is sterilised by passage through a multiple Seitz fitted with air sterilising pads. The platform with flask is placed in a large 37°C incubator. Other details of the apparatus may be seen in the diagram.

PRODUCTION

Small Erhlenmeyer flasks are inoculated from a Dorset egg slope and incubated overnight. Gram stains and blood agar plates are then prepared and further subcultures made into broth flasks. The following day, the large production flask is inoculated, if the Gram stains and plates are in order. During the preparation of

the inoculum and at least 24 hours before the large flask is inoculated, the apparatus is set up with rocker, gas, and incubator turned on. This provides the sterility check on apparatus and gas supply. The CO_2-O_2 mixture is adjusted to flow at 10 ml per minute. The culture is sampled at 24 hour intervals during the first 72 hours and thereafter four hourly. pH. measurements are made on the samples which are also plated out and Gram stained. Assays are made on the Seitz filtrates of the samples for rabbit cell haemolysins. At some time between 72 hours and 96 hours with this method a peak will be reached in haemolysin production. When the assay indicates that the peak has been reached, centrifugation of the bulk culture is commenced immediately. This is followed by Seitz filtration using a small addition of diatomaceous earth as a filter aid. The area of filter used must be carefully related to the volume to be filtered if losses of toxin by adsorption are to be minimised.

After sterility testing, the pH. of the filtrate is measured and it is assayed again for haemolysin titre. Toxoiding is then commenced by the addition of 0.2% formalin. This is usually complete in ten days when carried out at 37°C. Haemolysin assays are again carried out and when a uniformly low level is obtained conversion of toxin to toxoid is complete. It has been shown that when the logarithms of haemolytic titres are plotted against time a straight line graph results showing the loss of primary toxicity. Knowledge such as this of great value in practice in that it predicts the practical course of toxoiding procedure and permits the incorporation of a safety margin without unnecessary loss of antigenicity.

After sterility testing of the toxoid, safety tests are carried out on three species of animals in accordance with requirements of the B.P. The animal tests in conjunction with the haemolytic tests at the completion of toxoiding are designed to detect the lethal, dermonecrotic, and haemolytic factors and to assure that these substances have been toxoided during processing.

A suitable bacteriostat or preservative, usually 0.01% merthiolate, is added.

STANDARDISATION: vide B.P.

Not fewer than nine healthy guinea pigs (weighing not less than 250 gm each) which have not previously been treated with any antigenic material, are all given 1 ml subcutaneously on each of two occasions separated by an interval of not more than four weeks. Not later than two weeks after the second injection the serum of at least two-thirds of the guinea pigs tested must contain

at least 0.5 unit of staphylococcus alpha antitoxin per ml; alternatively the serum of at least one-third of the guinea pigs tested must contain at least 1 unit per ml.

The content of antitoxin in the serum may be determined by comparing the amount necessary to neutralise the haemolysis produced by uncombined toxin in 24 hours in a suspension of rabbit erythrocytes with the amount of the Standard Preparation of staphylococcus alpha antitoxin necessary to give the same degree of neutralisation.

Marketable biological products such as staphylococcal toxoid when prepared by commercial organisations are usually subject to State control through the Division of Public Hygiene of the Department of Health.

Control takes different forms in different countries but common to each system is the Protocol. This is a chronological record of every step made in a production schedule and contains all the relevant batch data. Considerable importance is attached to the protocol which is forwarded to a State Control Laboratory along with a sample of the product. Release of the production batch for bottling and distributing follows approval of the protocol and completion of official tests for identity, purity, safety, and potency.

SUMMARY

The history of the discovery and identification of the toxins of the Staphylococcus is reviewed with special reference to developments in laboratory production of high titre alpha-toxin.

The application of this knowledge to the commercial production of toxoid is described.

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ASPECTS OF WATER BACTERIOLOGY

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INTRODUCTION: Water bacteriology forms a small part of the work of many hospital laboratories. Most laboratories are too busy with the more pressing needs of the department to be able to give much time to the subject. Very often this work is given to the most junior members of the staff. In this paper I wish to bring before you some of the problems encountered in the Bacteriological Examination of Water.

METHOD OF DETERMINING THE PRESENCE OF COLIFORM ORGANISMS

Tube dilution:

The coliform group are defined as those organisms capable of fermenting lactose to form acid and gas. Any methods for their detection utilize this property. The member of the group

which is indicative of faecal contamination is Escherichia coli type I. The tube dilution method detects the most probable number (M.P.N.) of coliform organisms in 100 ml. of water. The confirmation tests then distinguish the different members of the group.

British workers use MacConkey broth and the American either lactose broth or lauryl tryptose phosphate broth.

Most laboratories in New Zealand probably follow the British standard and use MacConkey broth. Our laboratory has for a few years adopted the American standard. Johannesson (1957) working on surveys in Wellington Harbour showed that lactose broth was superior for the isolation of E. coli from sea water. Before being cultivated on inhibitory media, E coli from sea water needs a resuscitation period of a few hours on an enrichment medium. Allen and Childs (1953) showed that a resuscitation period was necessary with E. coli isolated from any polluted water. The method they proposed was to incubate the water sample in nutrient sugar broth for 1-2 hours at 37°C and then add the MacConkey broth of required strength to the tubes.

One of the main disadvantages of MacConkey broth is the variability of peptone and bile salts. A reliable brand of peptone must be used. The various brands of bile salts differ in their inhibitory power. Bile salts can be safely used in excess before showing any increase in inhibition. The good quality brands show optimum inhibition between 0.1 and 0.2 gm. %. The 0.5 gm. % MacConkey gave in his original media allowed quite a good margin but with some of the poorer brands an even higher concentration may be required. To use a random quantity such as 0.5 gm. % is both costly and risky. Burman (1955) stresses the need to standardize each new batch against a satisfactory bile salt kept as a standard. Variations in the inhibitory power can occur from batch to batch.

Jameson and Emberley (1956) substituted 0.1% teepol for bile salt in MacConkey. The teepol precipitated neutrol red so brom cresol purple was finally used as an indicator. The advantage of teepol was that it showed no variation from batch to batch and ten different batches were used. Teepol inhibited gram positive organisms and prevented the spreading of Proteus.

The search for chemically defined media led Burman and Oliver (1952) to compare Folpmer's glucose glutamic acid media with MacConkey broth. They also substituted lactose for glucose. Their findings and those of subsequent workers P.H.L.S. (1953) showed that lactose glutamic acid broth was superior to MacConkey broth in yield of E. coli type I, but had a moderately inhibtory effect on other coliform organisms. Glucose glutamic acid in-

creased the number of false positives. Although neither of these media could replace MacConkey the ultimate solution may lie in some modification of these media.

Confirmations of E. coli type I:

The most used tests for the confirmation of E. coli type I are the production of gas from lactose at 44°C and the production of indole. McKenzie, Taylor and Gilbert (1948) showed the value of including indole production at 44°C. E. coli type I is the only member of the group that gives positive reactions for both tests. Some hospital laboratories still keep the old routine of plating from MacConkey and performing the I M Vi C reactions (indole, methyl red, Voges Proskauer, and citrate utilization). This is tedious and time consuming, the first results being out in six days whereas using the 44°C bath results can be out in half the time. The accuracy of the bath is important and must be within 0.25°C. The tubes are incubated 6-24 hours. Brilliant green bile broth is used for fermentation of lactose at 44°C. This eliminates any false positives due to gram positive lactose fermenting bacilli. In our laboratory we use MacConkey at 37°C brilliant green bile broth at 44°C and indole production at 44°C as shown below.

Differentiation of Coliform types:

Gas in brilliant green bile broth at 44°C.	Indole production at 44°C.	
+	+	E. coli type I
+	—	Irregular type II Irregular type VI
—	+	Other
—	—	coliforms

Irregular type II and VI are not often found in human and animal faeces in England. Irregular type VI occurs in hemp and jute yarn and has been isolated from the faeces of humans in the East. If further identification is necessary then the I M Vi C reactions are used.

Effect of peptone on gas production from lactose at 44°C:

McKenzie, Taylor and Gilbert (1948) used Bacto peptone in their original media. Burman (1955) reports that false negative results were obtained using Bacto peptone. He investigated the problem from three angles, the size of the inoculum, comparison with MacConkey broth and the comparison of different brands of peptone.

He found that tubes giving positive results on the first culture will give a positive reaction after subculture on to solid media.

This suggested that the size of inoculum might be the influencing factor. He repeated his tests using a loop as before and a drop from a pipette. This resulted in a few more positive tubes but did not seem to be the only factor influencing the results.

Comparing MacConkey and brilliant green bile broth, MacConkey gave a higher percentage of confirmations than brilliant green, even when false positives were excluded. MacConkey contained English peptone and brilliant green bile broth American peptone.

This led to comparing Evans and Bacto peptone. Evans peptone reduced the number of negative tubes. We have found that Bacto peptone gives fewer positive tubes than Oxoid. As a routine we use Oxoid peptone in the brilliant green.

Different peptones and indole production:

We use Bacto tryptone and find that it gives consistent results. Burman found that the addition of 0.03% tryptophane to peptone water enabled good reactions to be obtained with Evans peptone and Oxoid peptone, but there was no improvement with tryptone. Tryptone gives good positive results at 37°C.

It appears that Evans peptone and Oxoid peptone contain some essential nutritional factor which is lacking in Bacto tryptone and is necessary for the production of indole at 44°C but not at 37°C. For this nutritional factor to be fully effective tryptophane is required.

The membrane filter method for determining the presence of E. coli type I:

Both American and British workers are considering using the membrane filter method for estimating the number of coliform organisms present in water. The required quantity of water is passed through the filter which is then placed on a pad saturated with culture media. For resuscitation it is necessary to place the filter on enrichment media for 1-2 hours before placing it on selective media. The pad is then transferred to selective media and incubated 16-20 hours. During incubation the pads must be kept moist. Moist filter paper in the lid of the petri dish is satisfactory.

The American practice given in Standard Methods (1955) suggests Difco M enrichment media for the resuscitation period. For selective media they recommend modified Endo media. This does not distinguish E. coli type I from the other members of the group as they all give a metallic sheen. Modified Eosin Methylene Blue has been used by some workers, Task group (1953). This media does distinguish the different types of colonies. In using this media the membranes should be pre-dyed by gently

boiling for ten minutes in 0.5% solution of methylene blue. Excess dye is removed by boiling in two changes of water for five minutes each.

The English practice differs from the American. Yeastrel broth is used for resuscitation. Two membranes are used for each sample one of which is incubated at 37°C and the other at 44°C. Modified MacConkey is the selective media. The plates incubated at 44°C are placed in sealed tins. All yellow colonies are counted, this must be done whilst the filters are still warm as the colour fades on cooling.

The merits of the method are:—

(1) The speed with which it can be performed, the results being obtainable in 20 hours. (2) Simplicity of the method and the removal of some errors such as those involved in dilution technique, etc.; with heavily polluted water less sample may be passed through the filter and one initial dilution is sufficient. (3) In pure water supplies large quantities can be handled.

Rapid presumptive test for coliform organisms (Levine et al, 1957):

As in the M.P.N. method this test is based on the detection of gas formed when coliform organisms ferment lactose. In this case radioactive carbon dioxide is formed and can be detected in very small quantities, hence the speed of the test. Brilliant green lactose bile broth is used to which is added 0.01% radio-active sodium formate. The water to be tested is passed through the membrane filter which is transferred to a planchet containing the culture media. These are incubated three hours then a planchet containing the barium hydroxide is added to the jar. Evolved radio-active carbon dioxide diffuses to the barium hydroxide and is precipitated as barium carbonate. After an hour the planchet is removed and dried and the amount of radio-active carbon is detected.

STREPTOCOCCI

Streptococci are not routinely used as an index of faecal pollution, but only when E coli type I results are inconclusive. With the use of membrane filters it seems that faecal streptococci may be used more as a means of detecting pollution. Faecal streptococci are not found in pure waters, virgin soil and sites out of contact with human and animal life, but are found in faeces, sewage and polluted waters. Faecal streptococci die out quickly but not as quickly as typhoid bacilli.

Slanetz, Bent and Bartley (1955 and 1957) give a media for use with the membrane filter. This media contains sodium azide and ethyl violet as inhibitory substances. Triphenyl tetrazo-

lium chloride was used to colour the colonies. The streptococci colonies on this media are red or pink. The membranes need to be incubated 48 hours before typical colonies develop. The results show that the method is far superior to that of the M.P.N. Much higher figures for enterococci in animal and human faeces are given with this method than before.

Cooper and Ramadan (1955) have worked on the differentiation between animal and human pollution by means of faecal streptococci. They compared three methods of isolating streptococci from human, bovine and sheep faeces using tetrathionate broth, 1:5,000 potassium tellurite and 1:2,000 thallium acetate. Potassium tellurite proved highly selective in concentrating streptococci from human and animal faeces; the isolation from human faeces was 100%. They also studied the physiological reactions of streptococci isolated from human, sheep and bovine faeces. Ten different types were found of which five were variants of typical Streptococcus faecalis.

The percentage occurrence of faecal streptococci in human, bovine and sheep faeces:

	Incidence Percentage in each group		
Classification	Human	Bovine	Sheep
S. faecalis—typical	40%	—	—
S. faecalis var. zymogenes	13%	—	6%
S. faecalis var. liquefaciens	15%	14%	18%
S. durans	4%	—	—
S. bovis	1%	28%	—
Atypical faecalis I	15%	21%	—
Atypical faecalis II	9%	—	39%
Atypical faecalis III	—	17%	22%
Atypical faecalis IV	—	14%	—
Atypical faecalis V	—	5%	13%

The one human strain of S. bovis isolated differed from the bovine in that it failed to ferment starch.

Based on this they devised three tests for distinguishing streptococci from different sources. These were the reduction of Janus green in milk, withstanding 60°C for thirty minutes and resistance to 1:5,000 potassium tellurite followed by heat resistance.

The greatest value of these tests is that streptococci in Groups I and II are of human origin. Anything falling outside this group is of indefinite origin. There is scope here in New Zealand to expand this work to the animals frequenting our reservoirs where animal pollution is high.

Results for tests distinguishing S. faecalis of human, bovine and sheep origin:

Group	Janus green milk test	Heat resistance	Heat and tellurite resistance	% Incidence in the Group Human	Bovine	Sheep
Group I	+	+	+	100%	—	—
Group II	—	+	+	100%	—	—
Group III	—	—	—	1.5%	50.4%	48.1%
Group IV	—	+	—	8.8%	37.6%	53.3%
Group V	+	+	—	46.7%	16.7%	36.7%
Group VI	+	—	—	27.8%	44.4%	27.8%

Barnes (1956) distinguishes S. faecium from S. faecalis on its inhibition by 1:2,500 potassium tellurite and less reduction of litmus milk. She suggests that the atypical faecalis of Cooper and Ramadan may be S. faecium but 1:5,000 potassium tellurite was not sufficient to inhibit growth. She also showed that S. faecium does not reduce tetrazolium as readily as S. faecalis. Using an agar containing tetrazolium S. faecium colonies were colourless and S. faecalis deep red.

Clostridium welchii may also be used as an index of faecal pollution, but owing to its resistance this may not necessarily be recent pollution. Cl. welchii is valuless in chlorinated waters as the spores withstand the chlorination required for pathogens.

MISCELLANEOUS

So far I have dealt solely with the organisms indicative of human and animal pollution. There are other bacteria which concern the water bacteriologist. These are the organisms which produce taste and odour and those which cause deposition of iron in pipes.

Taste and odour are often attributed to certain algae such as Asterionilla, Anabaena and Eudorina. Recent work in America by Silvey and Roach suggests that the odour and taste is not due so much to the algae but rather to the actinomycetes which dwell on them. These workers have isolated species of Streptomyces, Nocardia and Micromonospora from such waters. In Wellington we have started isolating a surprising number of different Actinomycetes from the local reservoirs. Taste complaints are largely in the summer when the plankton count is high.

Desulfovibrio desulphuricans reduces sulphate under anaerobic conditions liberating hydrogen sulphide. This gives taste and odour to the water. The methods of isolating these organisms are based on the principle of precipitation of black iron sulphide formed from the reaction between soluble iron in the media and the hydrogen sulphide produced by the bacteria.

The iron bacteria are capable of utilizing iron in the water and depositing it in the form of hydrated ferric hydroxide on their mucilaginous surfaces. These organisms are great slime producers and give a reddish colour and unpleasant odour to the water. They also cause a lot of trouble blocking pipes, etc.

Sulphur bacteria. These organisms derive their energy from the oxidation of reduced sulphur compounds. They are found in waters containing sewage or any naturally occurring sulphur. These organisms form slimes and are possibly responsible for some tastes and odours.

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IDENTIFICATION OF SALMONELLA BRAENDERUP IN NEW ZEALAND

By C. S. SHEPHERD
(*Pathology Department, Waikato Hospital.*)

In November, 1958, we received in our laboratory a faeces specimen from a female patient in the Taumaranui Hospital. She had developed nausea and vomiting, with abdominal tenderness.

On examination of the specimen, the culture revealed a gram negative motile organism exhibiting the biochemical and serological reactions of a Salmonella.

SEROLOGICAL INVESTIGATION

The organism was agglutinated by all sera containing the somatic factor VI and by a single factor VII serum. We also obtained in the flagellar phase, agglutination with the factor eh in phase 1, but despite many passages through semi-solid agar, to which factor eh had been added, we were only able to obtain agglutination with the eh factor once more. With the suspicion that the organism could be S. braenderup, the culture was forwarded to the National Health Institute, Wellington, where confirmation of this was made.

There they found the interesting fact that the antigen e occurred in both flagellar phases. In phase 1 the culture was agglutinated by all sera containing e, and by a single factor h serum. In phase 2, it was also agglutinated by all en sera, and by a single factor z_{15} serum. The antigenic structure was then—6, 7: eh, enz_{15}, that of S. braenderup.

HISTORY

Investigation into the patient's history revealed the following facts—born in England, she had lived for a while in West Germany before going to Mexico.

While staying in Mexico City, she worked at an hotel. There on the night of 19th September, 1958, she had a drink of water from a tap in her bedroom about 10 p.m. There was a jug of special drinking water available in her room, but the patient thought it had been there all day and was not fit to drink.

About 7 a.m. the following morning, violent abdominal pain and dysentery began. Amoebic dysentery was diagnosed, and after treatment with sulphaguanidine the complaint cleared up.

Arriving in New Zealand on 12th November, 1958, the patient had only been here a week when she again developed the same symptoms. She was at this time employed as a maid in a New Zealand Government tourist hotel.

The culture of the stools revealed S. braenderup.

DISCUSSION

S. braenderup was first isolated by Kauffman and Henningsen (1938) from faeces of a patient with enteritis, and also from the patient's cat which subsequently died.

The next occurrence was in Onderstepoorte, South Africa, two cultures being identified by Kauffman (1937).

In U.S.A. between 1934-47, Edwards, Bruner and Moran failed to record it among 12,331 Salmonella cultures.

In a survey by Nancy Atkinson (1956) in Australia, it was not found among 3,340 Salmonella strains between 1944-56.

Dr Joan Taylor of the Salmonella Reference Centre, Colindale, London, states that in England the first case was in 1942; since 1952 the following identifications were made: in 1952 1 human case from Nairobi; in 1953 2 human cases from Dar es Salaam; in 1955 there were two human cases in England; in 1956 three human cases in England and one isolation from an English sausage; in 1957 1 human case from Nairobi and 1 isolation from South African frozen egg; in 1958 3 human cases from Nairobi and 4 human cases in England, 2 isolations from fish meal, 1 from dust in a sack cleaning plant and 1 from a turkey.

SUMMARY

The identification of S. braenderup from human faeces in New Zealand is reported.

ACKNOWLEDGMENTS

The author wishes to thank Mr S. Josland of the National Health Institute, Wellington, for valuable information supplied and his interest shown.

Also Dr. Kean, Taumaranui, and Dr. W. C. Davidson, Medical Officer of Health, Rotorua, for clinical particulars kindly supplied, and Dr. W. A. Russell, Pathologist, Waikato Hospital, for permission to publish this paper.

BOOK REVIEW

A TEXTBOOK OF BACTERIOLOGY

By R. W. FAIRBROTHER, T.D., M.D., D.Sc., F.R.C.P.

8th Edition Revised 1959. Published by William Heinman Ltd.

The eighth revised edition of A Textbook of Bacteriology gives an excellent introduction to the principles of Bacteriology without delving too deeply into masses of technical detail.

It is a book primarily intended for the education of students of medicine but is well worth a place in the range of laboratory textbooks.

The chapters are well set out and cover individual characteristics of the organisms, the course of infection, diagnosis, prognosis and chemotherapy.

Those dealing with viruses and chemotherapy have been extensively revised and the latter chapter contains information on antibiotics not found in other textbooks available at present.

This book is worthy of attention by those commencing training in laboratory practice.

B.W.M.

FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE, AUGUST, 1959

NATIONAL HEALTH INSTITUTE WELLINGTON

Examiners: Drs. J. O. Mercer, J. M. Staveley, R. C. Taylor.

August 25th, 1959

WRITTEN EXAMINATION

Time allowed: 3 hours.

All questions to be attempted.

1. Describe your method for determining the ABO group of a person. Discuss the irregularities which may be encountered and state what steps you would take in each case to establish the ABO group.
2. Write brief notes on any four of the following:
   (a) The counting of leucocytes in cord blood.
   (b) The effect of external temperature on the E.S.R.
   (c) The clotting factors present in the serum.
   (d) The relation of the reticulocyte to the stippled cell.
   (e) A suitable anticoagulant mixture for the collection of blood for transfusion.
3. The result of a blood urea estimation is so different from the figure expected that the result is considered incorrect.
   (The method employs urease digestion, zinc hydroxide protein precipitation, Nessler's reagent, absorptiometry, and standard ammonium chloride solutions.)
   LIST the possible causes of this error, technical and otherwise, indicating where possible the effect on the final result.
   Discuss briefly "quality control".
4. A laboratoy (1) frequently carries out estimations on substances present in reconstituted freeze dried sera of varying but known content; and (2) carries over sera from one day to the next and repeats the estimations.
   What is the purpose of these manoeuvres and what is it called?
   Write what you know of "standard deviation".
   Define, or interpret in your own way, Beer's Law.
   Criticise in general terms the use of calibration curves for a method of some complexity. (e.g., Urease Nesslerization method for blood urea.)
5. (a) Describe a biological method for determining human pregnancy.
   (b) How would you determine the toxicity of a strain of Cl. welchii.
6. Discuss the use of dyes and indicators in bacteriological culture media.

PRACTICAL 1

MICROBIOLOGY AND HAEMATOLOGY

Tuesday, August 25, 1959, 2.30 p.m.-5.30 p.m.

1. What is the concentration of organisms in the bacterial suspension provided. Give your answer in organisms per ml.
   (Red cell suspension provided.)
2. Identify cultures B. C, D.
   B = S. sonnei.
   C = Pneumococci and Haemolytic Streptococci.
   D = Haemolytic and Non-Haemolytic Staphylococcus aureus.
3. Report on the catheter specimen of urine A.
   Cultural examination is required.
   (Pus, RBC, protein, enterococci.)

4. Report on the haemoglobin, white cell count and differential together with a platelet count in blood specimen Z.
(Specimen was from a patient with Polycythaemia vera.)

PRACTICAL II

MICROBIOLOGY AND HAEMATOLOGY

Wednesday, August 26, 1959- 9 a.m.-12 midday.

1. Complete identification of cultures from previous day.
2. Identify items (A) to (F.).
   (A) Hydatid scolices.
   (B) Sweat plate.
   (C) Macro conidia.
   (D) Bottle of Nigrosin.
   (E) Bottle of Aniline blue.
   (F) Micro filaria.
3. Report on Sputum (A).
(A few tubercle bacilli present.)
4. Make a detailed report on the morphology of the red cells, the leucocytes and the platelets in films A and B. A diagnosis is not required.
(A Frequent marcrocytes, normal white cells but total white count decreased, decreased number of platelets. Smear was from a partially treated P.A.)
(B Immature granulocytes in all stages. Polychromasia, marked anisocytosis, giant platelet forms. Smear was from a case of polycythaemia vera in transition to myelofibrosis).
5. The blood specimens X and Y are patient and pilot tube respectively. X is a group O, Rh negative and Y is group O, Rh positive. Because of lack of O, Rh negative blood it is necessary to consider the use of Rh positive blood.
(Compatible in saline cross match.
Incompatible in albumin cross match.
Incompatible in Coombs' cross match.)

PRACTICAL III

BIOCHEMISTRY

Wednesday, August 26th, 1959, 2.30 p.m.-5.30 p.m.

1. Graded solutions Cu SO_4 provided.
Estimate serum protein on the sample provided. Indicate any abnormality of serum which would alter the true value.
Give principles of two other methods of estimating total serum protein.
2. Indicate the number of bands in the haemoglobin solutions provided and their position in relation to the bands of oxyhaemoglobin. Identify the solutions.
3. Name and give the use of the displayed apparatus. A line per item is all that is required.
4. Estimate the normality of the sulphuric acid provided. Give your reason for choice of indicator and clearly set out calculations and equations used.
Solution of Na_2 CO_3 provided and a selection of indicators.
Atomic weights, Na = 23 C = 12 O = 16 H = 1 S = 32.
5. Estimate the chloride content of the serum provided, using thiocyanate back titration method. Explain the steps involved. Give your answer in milliequivalents per litre.

OR

(a) Standardize the mercuric nitrate solution provided by titrating against 2 mls. of sodium chloride provided.

(b) Estimate the serum chloride on a Folin & Wu filtrate by Schales and Schales Method.

ORAL EXAMINATION

Dr. Mercer:

Subjects: Animal inoculation tests, in particular diphtheria virulence. Isolation and animal inoculation of Cl. tetani. Isolation of the tubercle bacillus. Preparation of media. Autoclave control. Histological methods. Preparation of intravenous solutions. Recording of laboratory results. Sensitivity testing. Principles of Wassermann and flocculation tests for syphilis. Properties of complement. Acid digestion mixtures. Biochemical estimations on cerebrospinal fluid. Estimation of protein in C.S.F.

Dr. Staveley:

Subjects: Preparation of Coombs' reagent. Incomplete cold agglutination. Thrombocytopenia. Cross-matching for exchange transfusions. Myelomonocytic leukaemia. "Missing" anti A or anti B. Foetal Hb. Interpretation of Rh phenotypes.

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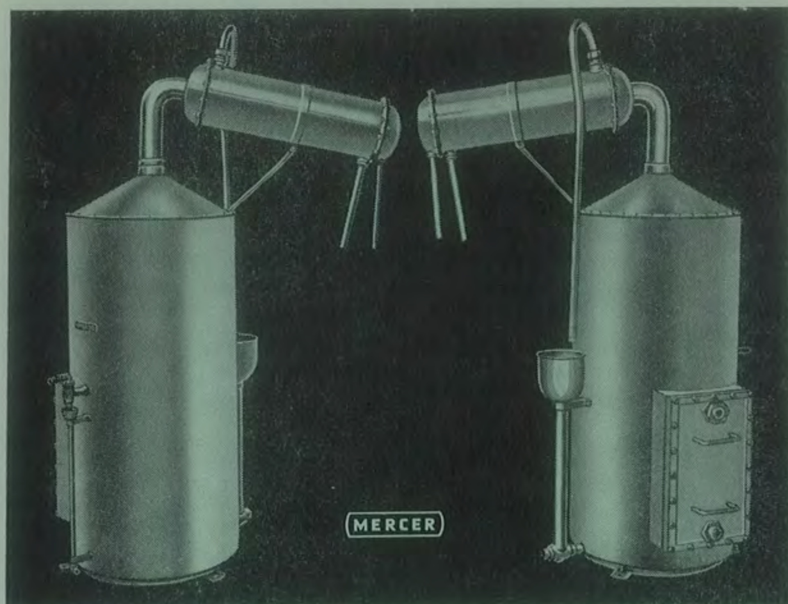

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