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NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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SOME DEVELOPMENTS IN LABORATORY DIAGNOSIS OF T.B.

By J. A. Samuel

Since my survey in this Journal of April, 1950, we have brought into use a number of improvements designed with the twin objects of increasing the recovery of organisms, and of decreasing the actual labour involved. I shall refer to the various steps in the same order as in my previous paper, adding finally, some improvements not previously referred to. This will facilitate reference to earlier statements.

ERRATA: There were errors in the previous survey (1) on page 28, first paragraph should read . . . . However, a bulky deposit is no *disadvantage* in a fluid medium . . .
(2) On page 29, 5th line from the bottom, for *Minimum*, read *Maximum.*

HOMOGENISATION: We now use an agitator consisting of a vertical metal disc, driven by a small motor through reduction gearing, at a speed of about half to one revolution per second. Spring clips are bolted to the disc to hold Universal containers radially. As we felt that continuous "slopping" overnight might be too drastic, we have plugged the motor into a Sunvic Simmerstat, which is set to switch the motor on for about ten seconds in every minute. The agitator is of course, mounted in the incubator. The slopping action will homogenise the most tenacious specimens.

Klaxon Type EK 1/20th H.P. Gallenkamp's Catalogue No. b-7579 with sliding resistance is a suitable outfit.

CONCENTRATION: During the past year, we have used alum in the saturated tri-sodium phosphate solution as a flocculant. We also suspected that the commercial tri-soda-phos contains alkali-resistant contaminants, so the solution is now filtered and boiled or autoclaved. Hoods over the siphon-tube tips are made from inverted clear plastic tumblers, held in place by rings of rubber tubing. The combination of sterilized alkali, and efficient homogenisation has reduced the overall contamination rate to one quarter. As the tri-soda-phos has a strong buffering action, the end-point on neutralisation is not easy to overshoot. If the tip of the siphon tube carrying the 25 per cent. HCl is made a suitable size, and the acid is allowed to shoot into the alkali in short,

strong spurts, this mixes the alkali and acid sufficiently to obviate shaking to determine whether neutrality has been reached. Note: When neutralising specimens containing a large proportion of protein, such as pleural fluids and some sputa, it will be noticed that there is a copious precipitate of protein where the solution is too acid. An indicator which would give a definite indication when the natural point is overshot would be an advantage. We have used a mixture of Brom-thymol blue and Phenol red for this purpose. This indicator gives a dirty purplish tint at neutrality and a definite yellowish on the acid side.

Alum flocculation without too much protein precipitation can be obtained if the end-point is made on the alkaline side, i.e., when the indicator is a distinct green, and the bottle is shaken immediately.

After neutralisation, the bottles are left for several minutes, for flocculation to take place, and for the floccules to settle. The tubes are now centrifuged for 5 minutes. It is not necessary for centrifuging to be prolonged, as the purpose is merely to pack the alum deposit. The floccules have already carried down the T.B. We have done an experiment to check this. A suspension of M. phlei was first centrifuged lightly to remove gross particles, and then centrifuged for up to 80 minutes, at an R.C.F. of 2,000. Samples of the supernatant and of the deposits were taken at 5, 10, 20, 40 and 80 minutes. It was found that, although, after 80 minutes 90 per cent. of the suspension had been deposited, there were still many bacilli to be found microscopically in the supernatant, and this supernatant had a distinct turbidity. In addition, there were many rafts of bacilli floating on the surface. After alum flocculation and 5 minutes centrifuging, the supernatant was optically clear to the naked eye, and no bacilli could be found microscopically either in the supernatant or on the surface.

Microscopy: A little care in setting up suitable microscope lamps is amply repaid in reduction of fatigue, and better definition, with fewer artefacts in the image. I have always considered that the ideal source for visual routine microscopy is a large, diffuse source placed close to the microscope. This gives a field which is evenly illuminated and absolutely free from diffraction effects round any object on the slide. For monocular microscopes, a pearl or opal bulb of about 60 watts is amply bright enough, but with binocular microscopes, no normal lamp will give sufficient brightness at magnifications of 1000. We have recently made some measurements with under run "Photolita" lamps, and have found that, for examining Z.N. stained slides, the *brightness* is at least as important as the *colour* of the light, in giving the most desirable picture. Wartime work on the physiology and psychology of seeing, especially with regard to optimum illumination intensity, has indicated that an illumination of about 100 foot candles is necessary for "prolonged critical seeing," even when the contrast is fairly high. We found that, using a Photolita 250 watt lamp running at an input of about 135 watts (i.e., using a dropping resistance made up of a 150 watt and a 200 watt lamp in parallel) we were able to attain an illumination in the image of 500 foot candles at a magnification of 1,000. This was

reduced by a smear of the usual thickness counter-stained with Loeffler's methylene blue, to about 60 foot candles. It is a simple matter to regulate the intensity of the Photolita lamp by changing the lamps run in series with it. If a sliding resistance of about 300 ohms to carry one amp. is available, this could be used with advantage to regulate the light to the intensity required for any magnification. Always start the lamp with maximum resistance in circuit, as this will prolong the life of the lamp considerably.

Summarising, a 250 watt mirror-backed "Photolita" lamp underrun at inputs of 100 to 130 watts gives as good illumination for visual work, as the special lamp described by McCartney, is much cheaper, and has a long life.

Z.N. Staining: In recent years, we have been rather dissatisfied with the intensity of staining of T.B. using the usual Z.N. Carbol Fuchsin. The effect may be due to different makes of stain, but a trial of Kinyoun's Carbol Fuchsin demonstrated that the stain could be strengthened with advantage.

Kinyoun's Carbol Fuchsin

Basic Fuchsin	40 gm.
Phenol	80 gm.
Alcohol	200 cc.
Water	1000 cc.

This stain gives a more beaded and thicker appearance to the bacilli, and the intensity of staining is very much greater. It has the disadvantage, that it dries on the slide rather quickly, and is then difficult to remove. However, if staining is carried out for the bare five minutes with heating, and any drying out is counteracted by adding a little more stain, there should be no difficulty. It is best to wash the slides in running water until free of all surplus stain, before decolourising. Similar concentrated carbol fuchsin stains containing glycerol, although not drying out, have given, in our hands, no better results than the normal Z.N. stain. See Pottenger ([7]) for a thorough discussion of stains and counter-stains.

C.S.F.: A method of centrifuging organisms directly onto a slide was described in W.H.O. Bulletin([2]). We have made this piece of equipment, and have tried it out on several positive specimens. We find that this method is at *least three times as effective* as the usual method of centrifuging in a small tube and making a smear with a loop. One great advantage is that the resultant deposit is very compact, and has a definite edge, beyond which one need not search. In addition, the cells lie close together, but not overlapping, and their morphology is unharmed. The overall result is that the deposit is much more satisfying to search. Another advantage is that the deposit contains practically no salt, compared with the smear made in the usual way. There is little likelihood, therefore, of organisms being washed away in the staining procedure. Since the deposit is only about 1/4 inch in diameter,

it is possible to search the *entire deposit from 5cc of C.S.F.* in about three hours. (Using a 1/7th inch oil immersion objective.)

At the lower powers allowable when searching by F.M. the time for search of the entire deposit could be reduced to 30 minutes. As experience with the original design indicates several improvements, a full description will be published later.

FLUORESCENCE MICROSCOPY: In the past two years, the following conditions have been found desirable —

1. Optimum magnification for single organisms 200. ($\frac{1}{3}$rd inch objective and x 10 eyepiece)
2. Optimum magnification for even small clumps of, say, 5 bacilli, may be as low as 50. (1in. objective and x 10 eyepiece).
3. Illuminants: By measuring the brightness of a gelatine screen stained with Auramine, when illuminated by the light passed through 1 cm of Cupramonium containing 5 per cent. $CuSO_4$, a Factor of Merit has been determined for the following light sources recommended by various authors.

	500 Watt	125 Watt	250 Watt
Lamp	Filament	Mercra	M.E. Box Type Y
Factor of Merit	1	2.25	5.6

As this test did not include the usual optical system, the 250 watt compact-source lamp is probably still more efficient, when this is taken into account. In the previous survey, I suggested that a number of 125 watt lamps might be arranged in such a way as to give a semi-dark-ground effect. After much experiment, I would say that it is definitely not worth while to attempt this. The result is not as good as that using the 250 watt M.E. compact source lamp.

4. Filters: Eyepiece filters—Ilford 110 (Micro 4 or Minus Blue)
   Ilford 111
   Wratten 15 (G)
   Wratten 16 (Flavazine T)

Any of these filters may be used in the eyepiece, with little apparent difference. Actual photometric measurement would give the advantage to Wratten 16. Any of these is far superior to Ilford Delta, 109.

Cupramonium filter. Matthei ([3]) and others have suggested diluting the filter solution progressively with ammonia until the background as seen through the microscope is a drab olive green. Diluting the solution further gives a rapid change towards a bright yellow-green against which the fluorescing organisms are lost. 1cm. thickness of Cupramonium containing 6 per cent. $CuSO_4$—$12H_2O$ is a good starting point, and will probably not have to be altered much.

5. Optics:—I still adhere to Kohler illumination, but Hughes at Waterfall Sanatorium, N.S.W., has the lamp very close to the microscope, with no intervening condenser.

One addition which I have found useful, is a patch stop under the microscope condenser. After maximum illumination is obtained, a patch stop is introduced, this being of such size as to reduce the intensity of the background without reducing the intensity of the fluorescing organisms. This patch stop can be of real value.

6. Stains:—The original Auramine stain is stable, and is used cold. The original Hughes Auramine—Rhodamine stain must be warmed to 70°C before use, and deteriorates if heated too much or too often. Warming to such a temperature is rather a nuisance. However, Matthei([3]) has described a stain as used at Melbourne University, which need be heated only to 55 or 60°C. This stain is stable. These stains containing Rhodamine, stain acid-fast organisms a reddish orange, while almost all artefacts stain a greenish yellow. If searching for single bacilli, it is almost essential to use a Rhodamine stain, to differentiate between acid-fast bacilli and artefacts. However, if searching for micro-colonies, the Auramine stain alone is sufficient as the clumps of acid-fast bacilli are brighter than most artefacts, have a characteristic shape (even in those strains which do not produce "cords"), and the colour of the fluorescing clumps is subtly different from that of artefacts, even when these are large and bright. I prefer the colour of Auramine-stained organisms, and so continue to use Auramine for work with micro-colonies.

I should emphasise here, the two different approaches to F.M. which have been used. The difficulty is that *single organisms in small numbers are lost among the artefacts if Auramine* alone is used. Hughes and others have overcome this problem by using Auramine-Rhodamine. This enables immediate recognition of T.B. among the artefacts.

After finding that micro-colonies only 4-7 days old (i.e., clumps of 4 to 20 bacilli) were easily distinguished from artefacts, even when stained with Auramine alone, I continued along the line: brief culture plus F.M. with Auramine at low powers. In my experience, the end-point at which T.B. are not found, is much the same whether the usual Z.N. stain is searched with a 1/7th oil or an Auramine stained slide is searched with a ⅓rd inch lens. This is entirely due to the T.B. being lost among the artefacts when Auramine is used. I have not yet been able to check up on the comparative efficiency of Z.N. and F.M. using the Auramine-Rhodamine stain.

7. *Fading of Auramine and Rhodamine*: Richards of American Optical Co. claims that the fluorescence of these stains is suppressed by infra-red, i.e., by the radiant heat from the lamp which is transmitted by the Cuprammonium filter. The suppression of fluorescence by infra-red is a well-known phenomenon, and as will be seen from the diagram in the previous paper the transmission of Cuprammonium is rising at 7,000 angstroms, and, indeed, rises steeply to complete transparency in the region of 12,000 Angstroms. However, I have shown that the fading of Auramine and Rhodamine is not due to infra-red, but

to the blue-violet light transmitted at the other end of the spectrum. In short, if a slide stained with Auramine-Rhodamine is left on the stage in one position for 5-10 minutes, it will be found that the fluorescence fades first to a yellow and then the yellow fades almost completely. Sunlight will also bleach the fluorescing bacilli, so keep any stock slides for testing in the dark. The normal fluorescence is regained on re-staining.

Culture Media: We continue to use Finlayson's Medium([4]) which has recently been reported to compare favourably with other media in the culture of Johne's bacillus.([5]) Many laboratories find difficulty in sterilising such medium in large batches. We have recently completed the construction of a steamer designed for this work.([6]) The principle on which the steamer works is very simple. By blowing air into the water at the bottom of the steamer, and thermostatically controlling the temperature of the water, it is possible to produce a vigorous stream of saturated air-water-vapour mixture at any desired temperature below 100°C. However, the stream of vapour must be lead horizontally between the trays of tubes in order to get the same heat input to all tubes. This method produces beautifully uniform batches of medium, which have undergone a minimum of heating under carefully controlled conditions.

CULTURING: Some laboratories use only one or two tubes of medium, while some use a two-ounce flat bottle, giving a large area, and pour the entire concentrate onto this. Up to March, 1952, we adhered to the suggestion of Finlayson and Edson that six tubes be inoculated, thus increasing the chance of picking up scanty bacilli, and decreasing the chance of contamination. We would welcome reports from laboratories using other methods, for comparison. The following is a brief analysis of our results over the past two years taking 1900 sputum specimens.

Positive on direct smear (Z.N. Stain, 1/7th oil imm)	358
Negative on direct, positive on concentrate	164
Negative on concentrate, positive on culture	193

Of those positive on culture:—

Number positive on all six tubes	34
Number positive on five tubes	28
Number positive on four tubes	21
Number positive on three tubes	22
Number positive on two tubes	32
Number positive on one tube	55

(Total number of specimens cultured 1,378, all negative microscopically. It will be noticed that one half of the cultures found positive, showed growth on only one or two tubes out of the six. The distribution of contaminants in the tubes showed a similar trend; but to a more marked degree:—of 432 cultures showing contamination.

Number contaminated on all six tubes	31
Number contaminated on five tubes	20
Number contaminated on four tubes	22
Number contaminated on three tubes	42
Number contaminated on two tubes	100
Number contaminated on one tube	217

These figures demonstrate without further analysis, the benefits of using a large inoculum, and spreading this over a number of tubes. The indications are that half of the specimens now found positive on culture would be missed, if only two tubes were inoculated. On the other hand, to obtain a significant increase in the proportion of positive cultures, it would be necessary to inoculate, say, twelve tubes, or increase the inoculum greatly.

We are at present running a trial in which three tubes are inoculated with approximately the same total amount of inoculum as has been used hitherto on six tubes.

Method of inoculating tubes: We have found that using a swab of *non-absorbent* wool on an applicator stick is a very successful method of inoculating T.B. concentrates. It is much easier to mix up the deposit with a swab than with a loop, and the swab allows more inoculum to be smeared evenly over the surface of the medium. Swabs may be sterilised one dozen in a large tube, one swab is used for each specimen, and then discarded. (This method was suggested by J. D. R. Morgan of this laboratory). A screw clamp set up to hold the centrifuge tubes in an almost horizontal position at a convenient height while inoculating, is a great time saver, as, once the top is removed, one has only the tubes of medium to handle.

Fluid Culture: The use of a fluid enrichment medium to which the entire concentrate might be added, followed by culture on solid medium, or by F.M. of the deposit was suggested in my survey of 1950 Preliminary experiments have given very promising results, but lack of time has prevented us from making a full investigation.

Cleaning Tubes: No greater protest arises from the Kitchen Staff than when confronted with several hundred tubes of egg medium to clean. *The following method reduces this work to thorough rinsing alone.*

After autoclaving the old cultures, stack them neatly in a four-gallon oil drum. Make up a solution containing:

10 per cent. Teepol

and 10 per cent. Tri-sodium-phosphate.

Pack one layer of tubes or bottles, pour in enough of the solution to fill, tilting the drum to ensure that all tubes are filled, and so on to the top. A 4-gallon oil drum will hold 1½ gross of universal containers. Leave the tubes in this for one week Pour off the solution (it can be used several times), and blow the now rubbery and soft medium out with a jet of water. Rinse thoroughly. *Note*: It is important that the

solution contains 10 per cent, of both teepol and tri-soda-phos. and that the tubes are left in the solution at least a week. If the solution is weaker, or if the tubes are taken out sooner, some of the medium adheres to the glass, whereas if the rules are followed, the medium may be tipped out of the tubes with a flicking action, or it may be forced out of bottles with a jet of water. The solution does not attack glass more than other alkaline solutions used for cleaning, and does not rust the drums.

This method will soften old serum or egg slopes which have hardened to a horn-like consistency.

Some people are alarmed by the possibility of wetting agent and alkali at such high concentrations remaining on the glass in significant amounts.

We have had no trouble—we even use alkali and teepol for cleaning W.R. tubes. (24 h. soak in 1 per cent. tri-soda-phos and 0.1 per cent. teepol followed by thorough rinsing in warm tap-water and 24 hr. soak in distilled water).

The following test was done on universal containers cleaned in the strong solution.

The bottles, after one week in the cleaning solution were rinsed quickly in 4 changes of warm tap-water and stood upside down to drain at room temperature in a wire basket. The following day they were filled with boiled distilled water and autoclaved. The distilled water pH, by glass electrode, was raised by only 0.1H unit after autoclaving. This should dispose of the prejudice against alkali and wettting agents in the cleaning of glassware. It should be noted that the classical chromic acid method was devised by chemists for cleaning tars out of retorts, when these tars resisted all available solvents. Since then, chromic acid has been invoked for many jobs where its use is not really necessary. To use chromic acid for large scale cleaning is to expose staff to unnecessary risks.

Other synthetic detergents should work equally well, if used in dilutions corresponding to their activity. Lissapol N, for example, should work at a concentration of about 3 per cent.

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(1) J.N.Z. Assn. Bact. Vol. 5 No. 2.

(2) Bull. of W.H.O. Vol. 3 No. 2, p258.

(3) J. Gen. Microbiol. Vol. 4, p393.

(4) J. Path and Bact. Vol. 58 p88.

(5) J. Path and Bact. Vol. 63 p333.

(6) J. N.Z. Assn. of Bact. Vol. 8 No. 2, p17.

(7) Am. Rev. Tuberc. Vol. 45, p549.

PENICILLIN 1928-1945:

Penicillin has become commonplace so that we accept its wonderful substance with no thought of the long arduous years spent in the many attempts to isolate, identify and produce the secretion of a mould.

Alexander Fleming, a Scottish doctor, worked at bacteriology at St. Mary's Hospital in London. He returned from a holiday in September, 1928, and while sorting staphylococcal culture plates which had been lying on the bench, he noticed a contaminating mould surrounded by a clear zone in a culture. Fleming was impressed and obtained filtrates of both cultures of the mould with which to experiment. His enthusiasm over the ensuing results was tremendous. The mould, Penicillin notatum was then wrongly identified by a mycologist as P. rubrum, a mistake unnoticed until 1931.

Fleming's work on penicillin in the three years after the discovery was extensive and he published several papers. He tried the penicillin filtrate on patients for irrigation purposes and found no ill effects. Yet, he failed to separate penicillin from the broth and even in 1940, he considered production impracticable owing to penicillin's unstable properties. Recently, when asked why he failed, Fleming said: "The final stages could be worked out only by the biochemist."

The chemical problems which Fleming could not solve chanced to interest Professor Harold Raistrick, a noted biochemist at London University in 1931. He had much experience in mycology and undertook chemical research with penicillin, assisted by a bacteriologist, Dr. Lovell. After studying Fleming's papers, they began work. Raistrick succeeded in growing penicillin on a synthetic medium which in later years proved most useful. Like Fleming, he worked to extract penicillin from gallons of fluid and although he found a method using ether, water and a lowered pH, it was of little use as the penicillin lost its potency. Unfortunately Raistrick's work ended after a year because his assisting mycologist met with a fatal road accident and his bacteriologist resigned.

Two efforts at extracting penicillin had failed by 1932. A third failed when Dr. C. G. Payne who had studied medicine under Fleming, gave up work on penicillin mainly because of the variability of the mould. However, Dr. Payne did have particular success in treating gonococcal eye infections with crude penicillin broth.

U.S.A. took up the challenge when Dr. R. D. Reid, of Pennsylvania State College, studied Fleming's and Raistrick's papers and conducted research into the production and mode of action of penicillin. He concluded that bacteria were not destroyed but merely inhibited. Like Fleming, Raistrick and Payne, Reid was unable to extract penicillin from broth. Nevertheless, he added his contribution to the valuable information already accumulated.

Seven years after Fleming's discovery, Dr. Howard Florey, a brilliant Australian, became a Professor of Pathology at Oxford

University. Florey's chief interest was work on lytic agents. In 1938 his assisting biochemist, Dr. E. B. Chain, came upon Fleming's paper by sheer luck. Neither Florey nor Chain had heard of it before, but were very interested, and after also studying Raistrick's papers, in July, 1939, they began work on penicillin where he had stopped.

Fortunately, the outbreak of war prevented Dr. N. G. Heatley, a specialist in micro-chemical measurement, from leaving for Copenhagen, and at the invitation of Florey, Heatley concentrated all his ability on the problem of penicillin. An unit of measurement was necessary and by using the porcelain cup method of assay on agar against a test strain of Staph. aureus, they determined a standard solution giving a zone of 24 mm. diameter. Thus, the Oxford unit originated.

After some months, Chain found that penicillin's instability could be overcome at low temperatures. In 1940, by using a modification of Raistrick's ether extraction method at low temperature, they extracted penicillin as a pinch of brown powder with 99 per cent. impurities. Then production became complicated by frequent failures of the mould but by May, 1940, Florey had proof that penicillin was a true chemotherapeutic substance.

The "Oxford team" was joined by Professor Gardner, bacteriologist, Dr. Jennings, pathologist, and Dr. Abraham, organic chemist. On 24th August, 1940, 'Lancet' published the famous paper "Penicillin as a Chemotherapeutic Substance." Soon afterwards Heatley constructed the first large extraction apparatus out of glass and rubber tubing, and production of penicillin continued on a small scale. Meanwhile, Chain and Abraham worked on the problems of purification and after many failures they obtained a purer barium salt of penicillin by using chromatography.

Early in 1941 penicillin was used to treat a patient dying of bacteraemia but unfortunately there was not enough penicillin in existence and bacteria won the battle. Florey's next move was an effort to obtain large-scale factory production of penicillin for use of war casualties.

War dominated England, so in June, 1941, Florey and Heatley took the mould to U.S.A. where the Department of Agriculture agreed to help. While Heatley instructed new workers, Florey managed to interest several chemical firms in penicillin production and returned to England in October, 1941, with promises of penicillin being sent to Oxford for clinical trials. By 1943, American companies were in full production.

Meanwhile an English firm was producing penicillin in a small quantity. The wheel swung full circle by August, 1942. Fleming's friend was dying of streptococcal meningitis and Florey travelled to St. Mary's Hospital, London, and successfully treated the case with penicillin. This caused Fleming to ask the Government to aid production of penicillin in England and soon afterwards several large companies were grappling with production problems.

But the actual constitution of penicillin was unknown until 1945, when. at Oxford, Dr. Abraham and Mrs. Hodgkins obtanied pure crystals of the sodium salt. And so, 17 years after that first mould on Fleming's culture plate, the crystalline structure of penicillin became known. In the same year, a Nobel Prize was awarded to Fleming, Florey and Chain, but the work of the others must not be forgotten.

This essay was awarded first prize in the Junior Essay Competition, 1953. We congratulate the author, Miss M. J. Grey ,of New Plymouth.

APPENDICITIS COMPLICATING SHIGELLA SONNEI

G. D. C. Meads

(Pathology Department, New Plymouth Hospital)

Shigella sonnei has recently been isolated in this laboratory from the surgical material provided by acute suppurative appendicitis. On first consideration it is difficult to understand why appendicitis as a complication of bacillary dysentry is not commonly described when it is quite well established in Salmonellosis. In both Salmonellosis and bacillary dysentery, ulceration and congestion occur, and judging from the presence of blood and pus in the stools, inflammation of the intestinal mucosa is more extensive in the latter disease.

CASE: Mr. G. was living in a house in which members had been ill with proven Shigella sonnei dysentery He had an attack of diarrhoea with a little abdominal pain, but not sufficient to incapacitate him. This subsided. Severe pain began 48 hours later in the central abdomen and later shifted to the right iliac fossa. He had been constipated since the onset and unable to eat but had not vomited.

Clinical examination on admission to the Stratford Hospital was typical of acute appendicitis. At operation, free serous fluid was found in the peritoneal cavity. The caecum was inflamed and oedematous and the appendix gangrenous.

Recovery was interrupted on the seventh day by a short febrile illness. At this stage a single specimen of faeces sent to this laboratory for examination for pathogenic organisms was negative. The patient was discharged well on the 16th day.

On the suggestion of the District Medical Officer of Health, the appendix was sent to this laboratory for examination.

Culture yielded a profuse growth of Shigella sonnei.

The histology was that of a typical gangrenous appendix with faecolith about the mid point of the organ and the lumen filled with pus and blood.

It was the pathologist's reported opinion that the diffuse inflammatory reaction resulting from the dysenteriae infection had served as a precipitating agent to induce the usual obstruction and necrosis in the appendix.

The experience of this laboratory is that the "Sonei season" begins in the late autumn. Flies have long been regarded as important agents in its spread, and it is at this period of the year that Musca domestica and Calliphora become noticeably sluggish in movement, contaminating

food over which they crawl. It was at this time of the year that the foregoing case came to light in the midst of an outbreak that had its origin in Auckland.

On the whole, very little work on the bacteriology of appendicitis is to be found in the standard text books. In the literature available, Shigella sonnei has not been described as being involved in appendicitis except for the work of Saffier (1953). Bacteriology of appendicitis usually reveals only the commensals of the large bowel because at its commencement, appendicitis is probably very seldom due to direct bacterial attack, but to ulceration or necrosis from mechanical factors or vascular occlusion.

Polyposis of the large bowel and local peritonitis perhaps resulting in stricture and obstruction, have been the result of Shigella infection of the bowel. The possibility therefore exists of acute appendicitis complicating Shigella sonnei dysentery.

ACKNOWLEDGMENTS

Dr. Dennis Allen for helpful criticism in preparation of this paper.
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Dr T. H. Lawrie, Med. Supt, Stratford Hospital, for acess to clinical notes.

REFERENCE

Soffier, British Med. Journal, July 4, 1953.

ACHLORHYDRIA TEST WITHOUT INTUBATION

The process of determining the presence or absence of free gastric hydrochloric acid, involving the passing of a tube into the patient's stomach is, to say the least, a time-consuming one. Intubation is a trying chore for the physician or attendant, an uncomfortable process for the patient, and not looked forward to by either party.

Recent medical research has produced a simplified method for this test, which does not require intubation. The patient is instructed to take a test quantity of quinine carbacrylic (diagnex, Squibb), by mouth. Based on the simple displacement of quinine in the indicator resin by the hydrogen ions of the free hydrochloric acid of the stomach, a urine specimen, collected two hours later, is then tested for quinine residual, by ultraviolet fluorescence, the amounts indicative of positive or negative results.

In a study[1] of 240 patients, tested both by intubation and the diagnex method, the results were the same in 227. In nine, free gastric acid was not consistently demonstrated by intubation, but was invariably predicted by the latter. In the remaining four, the results of the diagnex test were uncertain but a second test in two of these confirmed the findings obtained by intubation.

REFERENCE

[1]Segal, H.L.: Determination of gastric acidity without intubation; clinical evaluation of quininium exchange indicator compound M. Clin. North America 35; 593-602, Mar. 1951.

N.Z. ASSOCIATION OF BACTERIOLOGISTS CONFERENCE, 1953.

The Conversazione held in the Pathology Department of the Christchurch Hospital from 7.30 p.m. until about 11.0 p.m. on Thursday was attended by almost all delegates and by certain invited friends in scientific societies. Great interest and appreciation was shown of the exhibits, most of which were of a routine nature, of course. Supper was served at 10 p.m.

Beginning at the western end of the department—which has, of necessity, been of somewhat ribbon-like development, though found quite satisfactory—there was the Histology Unit.

This unit exhibited a collection of eight microtomes of historical and developmental interest from the 1880's to the present day, an electrolytic bone decalcifier, a gelatine embedding method for the demonstration of gross sections of lung pathology and a selection of fifteen slides of histologic and histo-chemical methods.

The Bacteriology Unit showed films of Actinomyces bovis, Leptospira pomona, an acid-alcohol-fast saprophyte in sputum culture; methods of sensitivity tests of 6 antibiotics, of neutralising concentrates of sputa, etc., for Tb., of the Paul-Bunnell test, of standardising records of work done, of minimising wastage of time and effort by use of a "movement-free" laboratory, of the effective use of the cold room, of animal management and cage steam sterilising. The Media Room showed modern methods of mass-media-production under 20-year-old conditions—which were shortly to be replaced. The organisation of a Syringe Bank and a display of special paper bags for syringes were featured.

Biochemistry gave demonstrations in the use of the Hartridge Reversion spectroscope, the ultra-violet lamp box for fluorescence examinations, the Maclagan (modified Lange) colloidal gold test, blood volume estimation method with Evan's Blue dye, paper electrophoresis machine, and the Beckman colorimeter and flame photometer.

The Blood Bank entrance effectively displayed a diagramatic layout of the Blood Transfusion Service in Christchurch, showing the clerical system and laboratory procedures from "donor to plasma." The Bank showed the preparation of Plasma from citrated blood, details of serological procedures, photographs and descriptions of intravenous equipment, "Our Range of Products"—samples of each type of intravenous solution made in Christchurch including M/6 sod. lactate, sterile absolute alcohol, 0.9% ammonium chloride—breakages of bottles in autoclaves due to micro-cracks caused by old brush ends, trypsin as a quick and efficient substance for removing gelatin film from X-ray plates.

An invitation was extended to the Industrial Development Division of the D.S.I.R. who exhibited a needle sharpener being made for this hospital, an electronic apparatus for detecting metal in tissue and an electro-stimulator shock treatment machine.

Haematology demonstrated platelet counting using siliconed syringes and the Rees-Ecker diluting fluid. A graph showing the effects of cortisone and splenectomy on the bleeding times and platelet counts in a case of thrombocytopenic purpura was shown with it. The method of Parpart et al for the erythrocyte fragility test. A chart showing a marked increased fragility in a case of acquired acholuric jaudice was shown with this and methods for preparing bone marrow films and Dale and Laidlaw's coagulation time.

NOTE: Further details of all methods and techniques may be obtained by writing to a member of Christchurch Pathology Staff.

DEPT. OF HEALTH
(Medical School, Dunedin)

Certificate of Proficiency in Bacteriology and Clinical Pathology

Examiners: Prof. D'Ath, Drs. Lynch, Watts, Pullar and Mercer.

Written Paper, 22nd Feb., 1954. 3 hours, 9.30 a.m. - 12.30

1. What are the dangers to be avoided in the transfusion of blood? Discuss fully the factors involved in blood transfusion mishaps.
   Explain in detail the role of the laboratory in ensuring safety in blood transfusion.
2. Outline the steps to be taken in determining the pathogenicity of an organism of
   A. The Corynebacterium group.
   B. The Clostridium group.
3. Write short notes on the laboratory procedures involved in determining:
   a. Serum calcium.
   b. Red cell fragility.
   c. The Coomb's test.
   d. Sedimentation rate.
4. Describe the steps in the bacteriological investigation of a sample of Spanish Cream suspected of causing an outbreak of food-poisoning.

Practical Paper A

23rd Feb., 1954. 9.30 a.m. - 12.30 p.m.

1. Report on the following specimens and in each case state briefly what further steps, if any, should be taken to establish a diagnosis. Smears, where given, are fixed but unstained: be careful of these as no extra ones can be provided.
   a. Two direct smears from a swab, and cultures (Loeffler, Blood agar and tellurite) from a case of suspected diptheria. (*This was negative—black colonies on tellurite were Gram positive cocci.*)
   b. Culture from a C.S.F. (*This was a meningococcus*)
   c. Hairs from a suspected ringworm (*Positive.*)
   d. Smear from fluid removed post-mortem from base of the brain. (*This was Cryptococcus neoformans.*)
2. Outline the steps of the procedure for determining quantitatively the bilirubin content of serum. Outline the principles underlying the test.
3. Outline the method of preparation of:
   a. The stains used for the identification of T.B. in a histological preparation.
   b. A suitable anticoagulant for the collection of blood for storage in a blood bank.
4. Outline two methods of preparing a protein-free filtrate from blood. Give one example of an estimation for which each is the more suitable.

Practical Paper B

23rd Feb. 2.30 p.m. - 5.30 p.m.

1. Write short notes on specimens 1-8 (2 minutes allowed for each).
   (*These were hydatid scolices, anthrax colony, H. influenzae in C.S.F., acid-fast bacilli, Ascaris ovum, Sarcoptes scabei, gametocyte of Pl-falciparum, ovum of Enterobius.*)
2. Examine the stained blood films and report on each one. Do a differential count on each one.
   (*a chronic myelogenous leukaemia and malaria.*)
3. Examine and report on the deposit in the specimen of urine provided.
   (*Red cells, occas, leukocyte and granular cast.*)
4. Name each piece of apparatus and indicate its use. Briefly indicate the basic principles on which its use depends.

1 *Van Slyke co2 apparatus; 2 pH meter (electric); 3 Seitz filter; 4 photo-electric colorimeter.)*

Orals

Some of the questions were as follows:—

Origin of platelets; significance of platelet levels; Saccharimeter; Gregerson's test for occult blood and the principle; differentiation of myeloblasts and lymphoblasts; phage-typing of Staphylococci and Salmonellae; Micrometer eye-piece and mircrometer slide; resolution; Measurement of depth of object under microscope by means of graduations on fine adjustment; Haemoglobin estimations; oxyhaemoglobin; Preparation of H.C.F. and G.C.F. antigens; Complement fixation tests; use of concave mirror on microscope; T.N.P.N.; Micro-Keljdahl; Parasites including Echinococcus, Ascaris, Enterobius, Taenia, and their life-cycles and transmission; spectrophotometers.

We offer our congratulations to the following candidates who were successful in this examination:—

Miss M. L. Grey (New Plymouth).
Miss J. R. Perry (Wellington).
Mr. G. R. Rose (Christchurch).
Miss S. J. Sinclair (Dunedin).
Miss B. A. Smith (Wellington).
Miss J. Sorenson (Wellington).
Mr. D. G. Till (Christchurch).
Miss A. L. Werry (Wellington).

There was no Intermediate Examination held in October, 1953, as there were insufficient candidates offering.

PHENOLISED THROMBOPLASTIN

Bacterial contamination is a common cause of rapid deterioration necessitating frequent preparation of fresh extract. Using phenol in the saline, I have found the extraction to yield a greater volume of thromboplastin and that decline of activity, although variable with the concentration, is usually from one to five seconds in six weeks. One particular batch showed no deterioration after six months. Commercial (Commonwealth) thromboplastin may be used.

Preparation methods 10 ml. of 0.5 per cent. phenol in 0.85 per cent. saline is added to 0.15 grams of thromboplastin in a dry test-tube. The mixture is heated 10 minutes at 45-48C., inverting twice, at five and ten minutes. It is cooled under tap and centrifuged for one minute at 800 revolutions per minute. Then it is decanted into a 100ml. flask and standardised until a standard time of 13 to 16 seconds is reached. Store at 4°C. Mix before using.

REFERENCE

M. Toohey; B.M.J. *i*, 518 (1950).

G. D. C. Meads,
Pathology Dept.,
New Plymouth.

ISOLATIONS OF INTESTINAL PATHOGENS

J. T. Connolly

(*Dept. of Pathology, Auckland Hospital*)

The following is a summary of the intestinal pathogens isolated during 1953 in the Bacteriology Department of Pathology, Auckland Hospital.

The routine method used for isolation from faeces was direct plating onto MacConkey agar, and enrichment in Selenite F medium followed by plating onto MacConkey agar. Biochemical reactions were performed on non-lactose fermenting colonies, suspicious organisms tested by slide agglutination and those giving positive reactions verified by tube agglutinations to the titre of the serum. Subsequent isolations from proved cases were tested by slide agglutination only.

The routine followed for blood cultures was initial cultivation in bile broth followed by plating onto MacConkey agar.

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

SALMONELLA TYPHI

Total No. of Cases	No. Times Isolated	Isolations Blood	Isolations Faeces	Isolations C.S.F.	Isolations Intestinal Ulcer
31	93	32	59	1	1

Average isolations per case 3.0.

SALMONELLA PARATYPHI A.

Total No. of Cases	No. Times Isolated	Isolations Blood	Isolations Faeces	Isolations Urine
21	50	14	34	2

Average isolations per case 2.4.

SALMONELLAE

Type	No. of Cases	No. of Times Isolated	Average Isolation Per Case
S. typhimurium	72	216	3.0
S. morbificans bovis	19	37	2.0
S. anatum	5	30	6.0
S. cholerae-suis	3	4	1.3
S. senftenberg	1	1	1.0

In one case of S. typhimurium no gas was produced on primary isolation, but after several subcultures, gassing did occur.

In another case, the organism was originally isolated from a throat swab and subsequently from faeces.

In a third case, the organism was originally isolated from urine and subsequently from faeces.

One case of infection with S. cholerae suis yielded the organism from both the blood and an osteomyelitis wound in the right shoulder.

SHIGELLAE

	No. of Cases	Isolated No. of Times	Per Case Average Isolations
Sh. sonnei	27	65	2.4
Sh. flexneri W	10	14	1.4
Sh. flexneri Z	1	1	1.0
Sh. flexneri 88	1	1	1.0
Total number of positive cases		191	
Total isolations		512	
Average isolations per case		2.7	

S. senftenberg, S. anatum and S. cholerae-suis were kindly identified for us by Mr. S. W. Josland of the Animal Research Station, Wallaceville.

DOMINION LABORATORY BOOKLET

A booklet recently published by the Department of Scientific and Industrial Research is devoted to the work of the Dominion Laboratory. Although primarily designed to acquaint the public with the work of this laboratory, the booklet is of considerable interest to workers in other scientific fields. The Dominion Laboratory may be termed the chemical section of the Department of Scientific and Industrial Research. It is surprising to learn of the wide scope of its work.

Although providing a service for a great many Government Departments, its main efforts are directed to the investigation and development of the country's natural resources. Various sections are devoted to the study of rocks and minerals, building materials, food and drugs, analytical chemistry, meat research, wood chemistry, paint, petroleum, coal and geothermal chemistry.

Members are reminded that Annual Subscriptions are now payable. They should be sent to the Treasurer:—

MR. R. J. PATTERSON,

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