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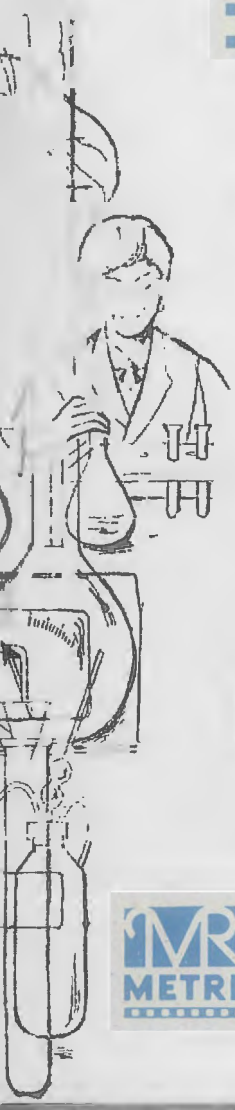

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Streptococcal Antihyaluronidase Titre
Method and Results Compared with Streptococcal Antistreptolysin Titre

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Dunedin

Received for publication, July, 1967.

The laboratory diagnosis of post-streptococcal infections — mainly rheumatic fever and acute haemorrhagic glomerulonephritis — falls into two sections, *viz:*—

A. The findings of raised erythrocyte sedimentation rate (ESR), elevated total white cell count (WBC) and positive C-reactive protein (CRP). These tests are of no differential diagnostic value, but where the diagnosis of rheumatic fever or acute haemorrhagic glomerulonephritis is established their results are roughly parallel, and are useful indicators of the presence or absence of disease activity.

B. The studies, bacteriological and immunological, that confirm a prior streptococcal infection. Cultures from patients with proven rheumatic fever or haemorrhagic glomerulonephritis do not usually yield positive streptococcal isolation by the time the disease process is manifest.

The antibody tests, on the other hand, help in determining whether the disease process is properly classified as acute rheumatic fever or acute haemorrhagic glomerulonephritis.

Antibody to streptolysin O (antistreptolysin or AST) has been measured as the classical method of studying the response to streptococcal infections in man, a rise in AST level being interpreted as reliable evidence of streptococcal infection.

Hyaluronidase or "spreading factor" was first extracted by Chain and Duthie[1] and the first techniques for the measurement of an antibody to the factor — antihyaluronidase or AHT — were published by Friou and Werner[2], Quinn[6], and Harris and Harris[3].

The *in-vitro* determination of AHT in serum is based on the specific inhibition of the capacity of streptococcal hyaluronidase to digest hyaluronic acid. Two techniques have been described, (1) turbidimetric and (2) mucin clot prevention. It is with the second method that this paper is concerned.

The test consists essentially of:

1. Combining a constant volume of standardised hyaluronidase with various dilutions of the patient's serum.
2. Incubating at 37°C. for 15 minutes and cooling in the refrigerator for 10 minutes.

3. Adding potassium hyaluronate, incubating at 37°C. for 20 minutes and refrigerating for 30 minutes.
4. Adding acetic acid and shaking vigorously.
5. Observing the tubes for the presence or absence of clot formation and clarity.

The AHT is expressed as the reciprocal of the highest serum dilution showing a clot (not a thread),[9] the dilutions used being 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048.

In this laboratory the AHT has been determined, using reagents supplied by Difco, on the same samples of serum as the AST. The AST has been determined by the standard method of Rantz and Randall[8] and expressed in Todd units, using reagents supplied by Baltimore Biological Limited.

The results of 500 duplicate determinations by the above method are listed in Table I.

AST TODD UNITS	Up to 32	64	128	256	512	1024	2048 or greater
2500 or greater	—	—	—	—	1	2	3
1250	1	—	—	1	2	2	5
833	1	—	—	—	1	1	4
625	1	1	1	1	10	8	7
500	1	1	2	1	3	6	2
333	8	9	12	15	20	7	6
250	7	6	12	13	18	4	4
166	14	19	21	26	4	2	2
less than 166	106	40	35	23	13	1	1
	AHT UNITS						

Table I showing results obtained on 500 duplicate determinations.

If we assume a normal AST on a casual blood specimen from a patient with no recent history of acute streptococcal infection of up to and including 250 Todd units, the majority of levels (86.8%) for the corresponding AHT go to 512 units.

At an AST level of 333 Todd units 57% of the AHT levels lie below 512 and 43% above. We feel confident of being able to say that an AHT of up to and including 256 units is normal, if any antibody level can be normal on the strength of one casual determination. A repeat estimation is advisable above 256 units.

No attempt has been made to break down the AHT level in relationship to age, but in a survey by Rantz, DiCaprio and

Randall[7] they suggest that AHT levels in young people (5-12 years) lie at a slightly higher level than young adults.

They hyaluronidase of the Group A streptococcus is immunologically distinct from the hyaluronidases of other sources, *i.e.* other groups of streptococci, other bacteria and tissue.[4,5] The other bacteria include *Staphylococcus aureus,* pneumococci, various strains of Clostridia and some strains of diphtheria bacilli.[11]

It will be seen from the table of results that a small number of normal AST sera gave elevated AHT results. On conferring with the medical practitioners, it was revealed that all these cases have a history of streptococcal throat infections with no further involvement.

We have noticed in the course of this work that the level of AHT rises more quickly than AST in proven post-streptococcal disease, and appears to vary more with the patent's progress. Three cases, with laboratory findings, are quoted.

Case Reports

Case 1. R.S., male 15.

First seen on 17.3.67, complaining of sore throat, swollen knee and swollen eyelids.

On examination the pulse was raised, temperature elevated, an apical systolic murmur was heard and tinea-like rash noted. Rheumatic fever was provisionally diagnosed and the patient given salicylates and complete bed rest.

On 22.3.67 the temperature and pulse were still elevated and the circinate rash had spread. The patient felt well.

On 25.5.67 the heart sounds had returned to normal, there was no further joint involvement, but the rash had spread still further and the patient exhibited a pronounced allergic reaction to an unknown allergen. Apart from this reaction the patient was well.

Laboratory findings:

21.3.67	ESR 17 mm/hour (Westergren), AST 333 Todd units, AHT 512 units.
30.3.67	ESR 21 mm, AST 625 Todd units, AHT greater than 2048 units.
28.4.67	ESR 7 mm, AST 625 Todd units, AHT greater than 2048 units.
30.5.67	ESR 10 mm, AST 833 Todd units, AHT greater than 2048 units, Total WBC 8,000/cmm. with 15% eosinophils.

Case 2. W.M-G., male, 21 years. Rheumatic fever.

First seen 27.2.67 with joint pain and swelling of fleeting character in several joints for 3-4 days, temperature elevated. The joint pains subsided within 10 days on salicylates. The heart was not involved and the ECG was normal.

Laboratory findings:

28.2.67	ESR 24 mm/hour, WBC 13,000 with a shift to the left, throat swab N.A.D., urine N.A.D., AST 166 Todd units, AHT 512 units.
6.3.67	ESR 49 mm, AST 250 Todd units, AHT greater than 2048 units.
8.3.67	ESR 19 mm, WBC 6,000, differential normal.
10.3.67	ESR 17 mm.
12.3.67	ESR 9 mm.
16.3.67	ESR 6 mm.

20.3.67 ESR 9 mm, AST greater than 2500 Todd units, AHT 1024 units.
29.3.67 ESR 5 mm, AST 1250 Todd units, AHT 512 units.
3.4.67 AST 1250 Todd units, AHT greater than 2048 units.
13.4.67 AST 1250 Todd units, AHT greater than 2048 units.

Case 3. R.V., male 11. Acute haemorrhagic glomerulonephritis.

First seen 13.2.66 with oedema and oliguria and a diagnosis of acute nephritis was made. The blood pressure showed an initial rise, returning to normal in 2 weeks.

Laboratory findings:
13.2.66 ESR 15 mm, blood urea 34 mgm%, AST 250 Todd units, AHT 512 units.
22.2.66 AST 625 Todd units, AHT greater than 2048 units. Urinalysis: protein small amount +, red blood cells 20-30/HPF, hyaline and granular casts 2-3/HPF.
9.3.66 ESR 11 mm.
19.5.66 I.V.P. normal.
12.9.66 AST 250 Todd units, AHT 256 units.

Conclusion

Following well-documented untreated streptococcal infections, AST responses can be demonstrated in 80-85% of cases.[10] The AHT has proved a very useful test in this laboratory as a supplement to the AST, and has increased the accuracy of studying the antibody response to streptococcal infection.

Acknowledgments

The author acknowledges the valuable assistance of Dr E. J. Williams and Dr C. S. Morrison for case histories, Miss M. Kirk and Mrs A. Sharp for technical assistance and Mr S. Downes of the Scientific Department, Kempthorne Prosser & Co., for ensuring a supply of reagent from Difco Laboratories.

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A Comparison of Two Methods for the Isolation of Salmonellae from a Sewage Purification Plant

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Central Laboratory, Auckland Hospital.

Received for publication, August, 1967.

Numerous methods have been developed for the isolation of Salmonellae from foods, fertilisers, natural water, sewage and faeces. Being predominently enteric pathogens, Salmonellae occur in relatively small numbers amidst vast numbers of non-pathogenic coliform bacteria possessing similar cultural characteristics. Selective media of various formulations have been produced, with the object of inhibiting the growth of non-pathogens, thus allowing the presence of Salmonellae to become more readily apparent. The selenite enrichment medium of Leifson[4] (1936) and its modifications Hobbs and Allison[2] (1945), North and Bartram[7] (1953), are widely used, as is also the tetrathionate broth of Muller[6] (1923) and its modifications.

Harvey and Thompson[3] (1953) reported that incubation at an elevated temperature in selenite broth increased the isolation rate of Salmonellae from faeces. Using a modified Moore swab, Spino[9] (1966) recovered Salmonellae consistently from surface waters when the enrichment broths of selenite brilliant green sulpha and tetrathionate, and brilliant green agar plates were incubated at 41.5°C. When an equal portion of the same swab sample was incubated at 37°C., no Salmonellae were detected. Salmonellae were recovered from stream sites having relatively low coliform densities of 2,200 per 100 ml. and faecal coliform densities of 220 per 100 ml. using the elevated temperature technique.

Stuart and Pivnick[11] (1965) described a method for isolating Salmonellae by selective motility (SM) systems using modified 'U' tubes and semi-solid enrichment media. Comparative studies showed that recovery of Salmonellae by SM techniques was equal or superior to that of standard procedures employed in two routine diagnostic laboratories. Primary isolations of Salmonellae organisms from SM enrichment were relatively free from normal faecal bacteria, often pure, and required only 15% of the media and correspondingly less time and skill than the usual methods for isolating Salmonellae.

The purpose of this paper is to present the results obtained using two different techniques for isolating Salmonellae from samples collected at various sites in the Manukau Sewage Purification Plant. Samples consisted of approximately 350 ml. of surface water collected at intervals over a period of several months from an oxidation pond, a sludge lagoon, and the outfall from an oxidation pond. The original culture procedure adopted was to add the sample to an equal volume of double strength selenite broth, followed by incubation at 37°C. for 18-24 hours and then plating

on to MacConkey agar. One isolation of *Salmonella typhimurium* was made from approximately 100 samples investigated using this technique.

It was decided at this stage of the investigation to substitute the Selenite Brilliant Green broth (S.B.G.) of Stokes and Osborne[10] (1955) for selenite broth and Salmonella Shigella (S.S.) agar with the addition of 1% sucrose used as an additional plating medium. It was also decided to investigate the use of the medium of Rappaport *et al.*[8] (1956) in this survey, as it had been shown to be superior to selenite and tetrathionate broths for the isolation of Salmonellae, with the exception of *Salmonella typhi*, from faeces.

Materials and Methods

Selenite Brilliant Green S.B.G. Broth.

The S.B.G. medium was prepared by weighing 2.4 g. of dehydrated medium into a sterile dry flask, adding 100 ml. of sample, and gently swirling the flask to obtain dissolution of the powder. Incubation was carried out at 37°C. overnight with minimal agitation of the flask during and after incubation. MacConkey and S.S. agar plates were inoculated with a loopful of culture from the topmost inch of the S.B.G. medium and incubated at 40°C. for 24 hours. This procedure follows that recommended by Livingstone[5] (1965).

Rappaport Medium.

Solution A		
	Bacto tryptone	5.0 g.
	NaCl	8.0 g.
	KH_2PO_4	1.6 g.
	Bi-dist. H_2O	100 ml.
Solution B		
	$MgCl_26H_2O$	75 g.
	dist. H_2O	100 ml.
Solution C		
	Malachite Green	0.4 g.
	dist. H_2O	100 ml.
Working Solution		
	Solution A	10 ml.
	Solution B	10 ml.
	Solution C	3 ml.

Solution A is ten times the concentration of the original formula to allow for dilution of the medium on addition of the sample. The medium was distributed in 250 ml. flasks and sterilised in the autoclave at 115°C. for 10 minutes. To each 23 ml. of working solution was added 100 ml. of sample, followed by incubation at 37°C. for 18-24 hours and plating onto MacConkey and S.S. agar. These plates were incubated at 37°C. for 24 hours.

Plate cultures from both enrichment broths were then examined, and typical colonies picked off on to phenylalanine agar slopes. Cultures which gave a negative reaction for phenylalanine

deaminase after 6 hours incubation were subcultured to Triple Sugar Iron (T.S.I.) Agar slants which were incubated overnight. Cultures which gave Salmonella-or Shigella-type reactions on T.S.I. were investigated serologically and biochemically. All Salmonellae isolated were forwarded to the National Health Institute to confirm or establish their identity.

Results

In a series of 16 samples, in which S.B.G. enrichment was used alone, 3 isolations of Salmonellae were made. A further series of 21 samples was investigated using both methods in parallel. The results of this series are recorded in Table I.

TABLE I

Method	No. of Samples	No. of Isolations
S.B.G.	21	4
Rappaport	21	13

During the period July 1965 to June 1966 a total of 52 samples were investigated. Table II lists the sites from which Salmonellae were recovered during this period with the number of isolations and serotypes isolated.

TABLE II

	Oxidation Pond	Sludge Lagoon	Outfall
S. schwarzengrund	1		1
S. derby		1	
S. typhimurium	1		1
S. brandenburg	1		
S. oranienburg	1		
S. infantis		1	
S. bareilly		1	
S. bovis morbificans	1	2	4
S. anatum	2	1	2
S. newington	4	1	

Discussion

Development of Rappaport medium was based on the findings of several workers who showed that when various bacteria were dried on filter paper, their sensitivity to dehydration differed. Proteus and *E. coli* were found to be more sensitive to drying than Salmonellae or Shigellae. It was shown experimentally that this property could be used for the differential isolation of Salmonellae from mixed cultures.

Rappaport and co-workers experimented with hypertonic solutions of various salts, since in them bacteria would be

dehydrated. Hypertonicity was found not to be the only factor, but that the ion used was also of importance. Of the various salts tried, $MgCl_2$ used at a concentration of 4% inhibited *E. coli* and Proteus to a degree, while Salmonellae were unaffected. Having established the optimal concentration of $MgCl_2$, varying concentrations of a number of different inhibiting dyes were added and their effect observed. Malachite green, the selective value of which was first demonstrated by Loeffler in 1903, was found to give the best results. Eleven different peptones were tried in the basal medium and Difco Tryptone was found to be superior for the growth of Salmonellae. The medium was found to be superior to selenite and tetrathionate broths for the enrichment of Salmonellae with the exception of *S. typhi,* which is inhibited by malachite green in the concentration employed.

Iveson *et al.*[3] (1964) reported an improved method of isolating Salmonellae from desiccated coconut using the medium of Rappaport *et al.* When used in parallel with selenite and tetrathionate media, significantly higher recovery rates of Salmonella organisms were made with Rappaport medium.

Stuart and Pivnick[11] (1965) used Rappaport medium incorporated in 0.6% agar in their SM system. A further modification was the substitution of brilliant green 0.001% for malachite green. Brilliant green was found to be less toxic and appeared to allow freer growth of all types of Salmonellae, whilst preserving most of the inhibitory action towards species that were not Salmonellae.

Conclusion

Although the number of samples investigated in this survey was a small one, we feel that the results obtained with the enrichment medium of Rappaport *et al.* are additional evidence of the value of this medium for the isolation of Salmonellae.

Acknowledgments

We wish to thank Dr H. D. Sutherland, Medical Officer of Health, South Auckland, for permission to publish the results of this survey, and Mr L. S. Horsman, Inspector of Health, for his assistance in this work.

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The Routine Bacteriological Examination of Urine Specimens

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In suspected urinary tract infection the bacteriological examination of a clean urine specimen may provide a valuable aid to diagnosis. However, if these examinations are not carried out under standardised conditions, their validity is questionable.

The purpose of this paper is to review the errors associated with the bacteriological examination of urine specimens, and to report on the methods which have been in use in this laboratory for the past six months, the results obtained from which have correlated well with the clinical findings.

The report on a urine sample should include:—

1. pH, protein, sugar and any other obvious deviation from the normal.
2. An accurate count of the cellular elements, casts and crystals.
3. A viable bacteria count.
4. Sensitivity tests on the pathogenic organisms.

Collection of Specimens

Clean midstream specimens are satisfactory for routine examination; in fact, they are preferable to catheter specimens, as catheterisation carries a 4-6% risk of introducing infection (Marple 1941[11], Brumfitt Davies and Rosser 1961[2],).

Midstream specimens from hospitalised patients are best collected after swabbing the glans or vulva with a cleansing solution. The literature quotes many such solutions, *i.e.*, Savlon, benzalkonium hydrochloride, saline, soap and water, or sterile water. In practice swabbing with soap and water, followed by a further wash with clean water is sufficient. This serves to safeguard against contamination of the sample with mucous secretions, without at the same time introducing antibacterial substances.

At the present, midstream urine specimens from hospital patients are obtained by the two-pan technique. However, in the near future it is proposed to introduce the collection system as described by Curtis 1964[5], utilising a clip-on container for the toilet. This method will simplify collection procedures.

The problem of obtaining suitable specimens from outpatients is well known. In an effort to achieve some degree of standardisation, outpatients at this hospital are given suitable sterilised containers, together with printed slips giving appropriate instructions for collection of the specimens.

All specimens are requested to be delivered to the laboratory as soon as possible, and they are cultured immediately on receipt, or stored in the refrigerator at 4°-6°C. for a maximum of four hours before examination.

The Effect of Urine pH on Bacteria and Cell Survival.

In acid urines (with a pH below 6.9), cells survive well; but as the urine becomes increasingly alkaline this survival time is shortened. At a pH of 8.4, cells disappear within a few minutes. (Stansfeld 1962[15],).

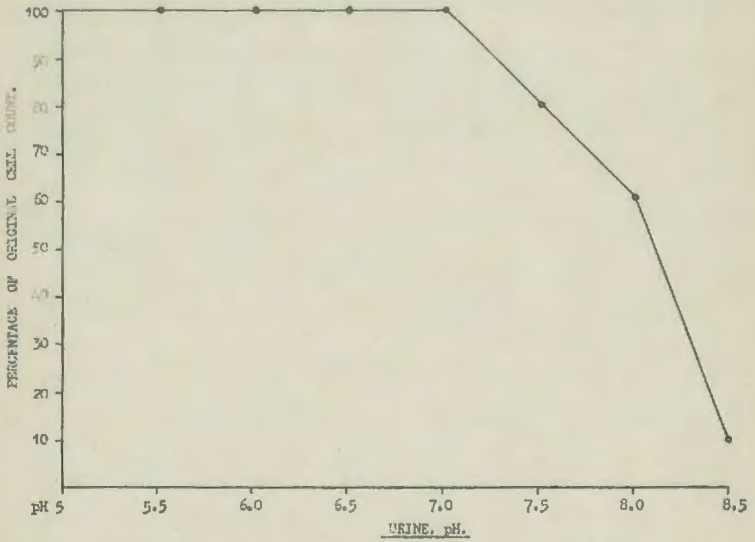

Figure 1. Graph illustrating the average decrease in leucocyte numbers in 10 urine samples, adjusted to varying pH levels.

Of 600 urines tested in this survey, 78 had a pH of 7.5 or greater. This would imply that 13% of the cell counts carried out on these specimens could have given erroneous results.

In urinary infection with Proteus organisms the urine is often strongly alkaline, and examination of the wet preparations of such specimens often shows debris only, or, at the most, leucocytes within normal numbers. For this reason all urine samples are cultured, regardless of the number of leucocytes present.

The effect of pH on the survival and multiplication of bacteria in urine prior to culture was also studied, and it was found that the pH values normally encountered in urine specimens had little effect on the multiplication of bacteria commonly found in urinary tract infection, nor was there any marked bactericidal power, provided the specimens were treated within the time limits as stated previously.

Culture Techniques.

Prior to the introduction of the system of urine examination described in this paper, all samples of urine received for bacterial examination at this laboratory were cultured using the centrifuged urine to inoculate the blood agar and MacConkey plates. It was found that this technique gave false positive results, and the cultures were difficult to interpret, as mixed growths of differing organisms were frequently found. In true renal infection it is rare for more than one species of bacteria to be causing the infective process. Because of these problems a standardised procedure was introduced for the assessment of bacterial numbers in the specimen.

Bacterial Counts.

The viable bacteria count has been well accepted as a worthwhile technique for the diagnosis of urinary tract infection, and a number of methods have been suggested for carrying it out, *e.g.*, "standard loop technique,[12, 6]" "pour plate[4]," "serial dilution[10]," "dip spoon[1]," "filter paper strip[13]," "reduction of triphenyl tetrazolium chloride[14]," "reduction of nitrite[16]."

Of these the standard loop technique was selected as being the most convenient for handling large numbers of specimens. It is more accurate than the chemical tests, trouble is not experienced with confluent growth, and whilst not as accurate as the more laborious pour plate or serial dilution techniques, the results obtained are sufficiently reliable for clinical purposes.

Method.

The standard loop is constructed from 22-gauge nichrome wire, and has an internal diameter of 3mm.

The specimens should be cultured in the following manner:—

1. Mix the specimen well.
2. Dip the loop into the specimen and remove vertically.
3. Inoculate the entire surface of a blood agar plate, using both sides of the loop, and inoculate a MacConkey plate in a similar manner after recharging the loop.
4. Incubate the MacConkey plate at 37°C. and the blood agar plate at 37°C. in an atmosphere of 5% CO_2.
5. Examine and count the number of colonies after 18-24 hours incubation.
6. Carry out sensitivity tests on those organisms showing significant numbers of colonies.

Interpretation.

When removed vertically from the sample, the standard loop contains approximately .0050 ml. of urine. Therefore, if 100 colonies are found on one of the culture plates the urine would have a count of 20,000 viable bacteria per ml.

100,000 bacteria per ml. is regarded as a significant bacteriuria (Kass 1956[9]).

However, lower figures may be encountered in true infection,

due to the presence of inhibiting substances in the urine, marked frequency, or the drinking of large volumes of fluid, resulting in the fact that the urine is not present in the bladder long enough for bacterial multiplication to have reached a figure of 100,000 per ml. (Brumfitt and Percival, 1964[3]).

In general, urine specimens with a count of over 10,000 organisms per ml. are infected, and we issue reports based on the findings of Brumfitt and Percival[3].

No of colonies of one type on plate	Viable Organisms per ml. of Urine	INTERPRETATION
0 — 5	0 — 1000	NOT INFECTED
5 — 50	1000 — 10,000	NO INFECTION (POSSIBLE CONTAMINATION)
50 — 500	10,000 — 100,000	PROBABLE INFECTION (SHOULD BE CONFIRMED)
500 +	100,000 +	INFECTION

Table I Illustrating the interpretation of significant bacteriuria.

Cell Counts.

If one is going to provide accurate bacterial counts of urine specimens, surely the cellular elements should be reported with equal precision.

The common practice of centrifuging "some" urine and examining "some" of the deposit in a coverslip preparation, then reporting cells per high power field leaves much to be desired, because of the many variables introduced.

These include: Variations in the degree of concentration by centrifugation, variations in the volume of urine used and variations in the depth of fluid under the coverslip.

The cell count carried out in a standard counting chamber is reliable. (Houghton and Pears 1957[6].) However, the process is time consuming and impracticable where large numbers of specimens are handled, owing to the difficulty in cleaning and sterilising the counting chambers.

We have developed a modified technique, using a disposable counting chamber based on the three coverslip method of Hilson (1964[7]).

If the specimen for examination has a neutral to acid pH the cell count obtained by the use of this technique can be reported with confidence.

Technique.

25 ml. of urine is centrifuged at an R.C.F. of 1500 for ten minutes in a universal container which has previously been marked at the 2.5 and 25 ml. levels. The supernatant fluid is withdrawn to the 2.5 ml. mark (using suction apparatus) and the waste is collected in a side-arm flask containing lysol. The deposit in the universal container is then well mixed and one drop of this is placed in the disposable counting chamber.

Disposable Counting Chamber.

Standard microscope slides are used and, at a distance ¼-inch on either side of centre, two strips of transparent self-adhesive tape are placed transversely (see Fig. 2). One drop of urine deposit is placed between the strips of tape (using the standard loop as described for bacterial counts) and a coverslip is then placed in position so that it bridges the gap between the two strips of adhesive tape.

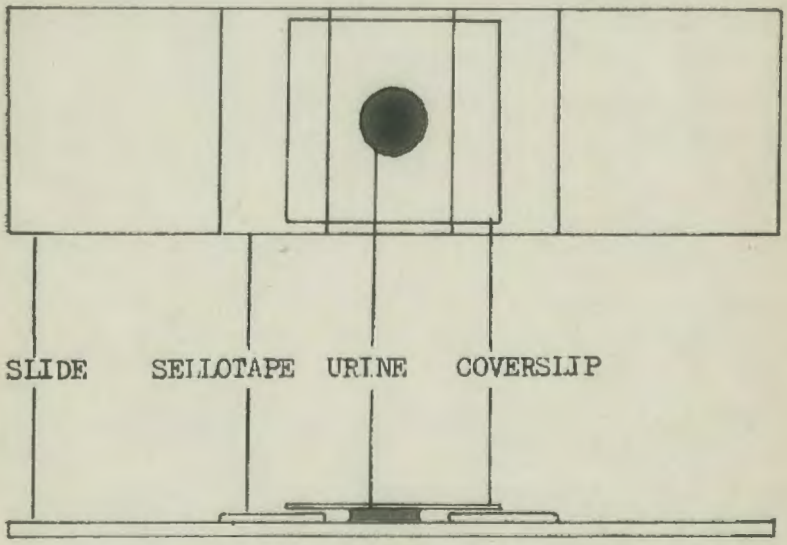

Figure 2. Slide as used for counting chamber.

After allowing a moment for the cells to settle, the cellular elements are counted, including casts and crystals, recording the average number per 20 high power fields.

Preparation of Slides for Use as Counting Chambers.

Large numbers of slides can be prepared with ease if the small jig (illustrated in Fig. 3) is utilised.

Standard miscroscope slides are placed in the jig and two strips of tape are stretched across the slides to make contact with the nails as illustrated.

The slides are then separated using a scalpel to cut the

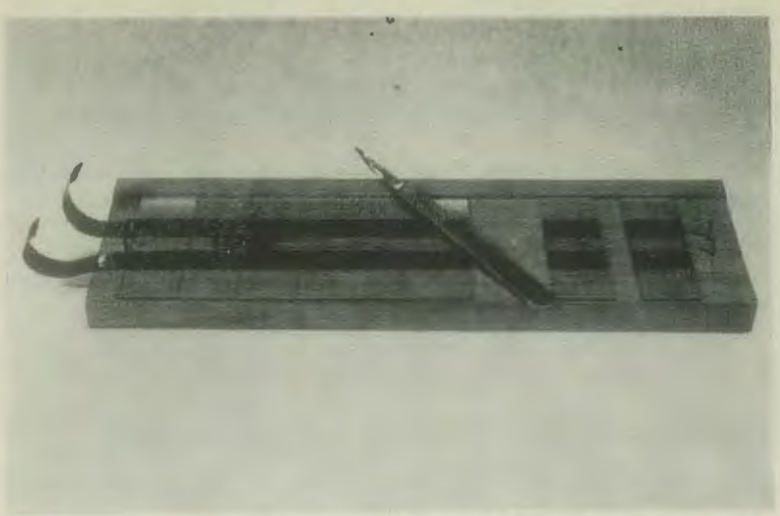

Figure 3. Jig used for preparation of slide counting chambers.

tape. Air bubbles, which sometimes form between the tape and the slides, are liberated if the slides are placed in a stack, under a weight, in the 37° C. incubator for some hours before use.

Calibration of Chamber.

The dimensions of the counting chamber can be determined and cells per high power field can be converted to cells per cmm.

By the use of a measuring slide the diameter of the high power field is found from this figure and the depth of the counting chamber is calculated the volume of urine in each field (ΠR^2 x depth = volume).

When using the high dry magnification on our microscope, the field diameter was found to be 0.365 mm. and the depth of the chamber governed by the thickness of the adhesive tape = 0.069mm.

Therefore, the volume of the cylinder seen as 1 H.P.F. = .007cmm.
and 1 Cell per H.P.F. = 143 per cmm.
or approximately 15 per cmm. allowing for 10 times concentration by centrifugation.

The chamber can also be calibrated using a cell suspension. A dilution of anticoagulated blood is made in an appropriate fluid, and cells per cmm. are counted using a standard counting chamber or an electronic counter. This figure is then related to cells seen per H.P.F. in the disposable chamber.

Both of the above techniques gave close correlation, and, finding that 1 cell per H.P.F. equalled approximately 15 cells per cmm. it was possible to fix a normal for the number of cells per H.P.F. (*i.e.*, not more than 3 leucocytes—equivalent to 45 cells per cmm.) 50 cells per cmm. is a significant pyuria (Brumfitt *et al.* 1961[2].)

Results.

This improved system of urine examination has resulted in a marked improvement in correlation between the laboratory and clinical findings in renal disease. Occasional discrepancies were found, but the examination of a second specimen from cases where there was doubt usually resolved any problems, *i.e.*, high bacterial counts with normal numbers of leucocytes are frequently caused by: Incorrect collection and storage of specimen, resulting in bacterial contamination and multiplication; alkaline urines, causing a reduction in cell numbers; and, rarely by apyuric bacteriurias.

Elevated leucocyte counts with normal bacterial counts are found in specimens from patients receiving treatment for infection or in any case where the urine contains antibacterial substances.

Discussion.

The cell count obtained by the use of the disposable counting chamber and the standardised urine concentration technique is reliable; and results obtained by this method compare favourably with counts performed in standard counting chambers. Some doubt arose concerning the advisability of attempting accurate cell counts on urine specimens when it was realised that urinary pH effected cell survival; however, on consideration it was found that most urine samples (*i.e.* 87% in our survey) had an acid to neutral pH, and would, therefore, be suitable for cell counts. Consequently we carry out counts on all specimens, but when the specimen has an alkaline pH, comment is made on the probable inaccuracy of the cell count.

The standard loop technique for bacterial counts made the interpretation of cultures a simple procedure, and the problem of deciding the significance of mixed growths, as are encountered when cultures are made from the centrifuged urine deposit, was not encountered. Single species of bacteria were responsible for most urinary infections, and in only 3.2% of cases in this survey was the infective process caused by more than one type of bacteria. The pathogen most frequently isolated was *E.coli* and this organism was present in 65% of all cases with proven urinary infection.

When the study of the effect of urinary pH on leucocytes was made it was felt that although pH was the predominant factor affecting cell survival, other considerations must be taken into account. It is hoped to examine this aspect of urinalysis more fully in the future.

A description of our technique for determining the chemical composition of the urine specimen has not been given in the text and a method of sensitivity testing is not detailed as it is felt that these procedures are relatively standardised in most laboratories.

Finally, the importance of receiving suitable clean specimens for the examinations cannot be over-emphasised. However, pro-

vided the laboratory receives co-operation in this respect, the techniques described should bring increased accuracy to our routine urinalysis. Obviously no one method of urine examination will meet with general approval; nevertheless, we feel that the routine described gives some standardisation to the tests, and provides a satisfying degree of reproducibility that is often lacking in other techniques.

Summary.

A simple method is suggested for the routine examination of urine specimens giving an accurate cell and bacterial count, by the use of a disposable counting chamber and a standard loop.

Acknowledgments.

I would like to thank Mr R. F. Hendtlass, Surgeon Superintendent, Oamaru Hospital, for permission to publish this paper, and I am indebted to Mr L. R. Taylor and members of the laboratory staff, who gave helpful advice and technical assistance.

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Aberrations in Continuous-flow Flame Photometry

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Introduction

Persistent, unexplained variations of the intermittent standards used for electrolyte determinations have caused concern in this laboratory. In an effort to improve the situation, the systematic investigations described in this article were undertaken.

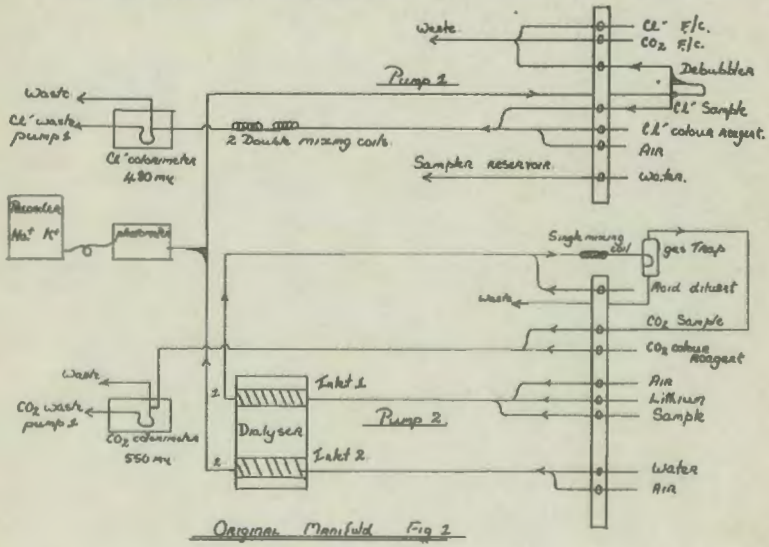
The results obtained by other workers in this field (Thiers and Oglesby 1964)[3] (Campbell and Annan 1966)[2] indicate that some variation is to be expected, but little has been published on the causal factors. On reflection there were many theoretical variables, and as a consequence a systematic elimination procedure was used, each assumed variable being thus discounted or incorporated in the final result.

Materials

Standard Technicon modules were used for the electrolyte determinations, the flow system being composed of:

1) Model 1 flame photometer, using oxygen propane at 10 p.s.i.
2) Bristol Model 570 2-pen recorder.
3) Model II Sampler running at 40 tests per hour.

The manifold currently in use is that shown in Fig. 1, which



Original Manifold Fig 1

allows simultaneous sodium, potassium, chloride and carbon dioxide estimations, using two pumps. All reagents are as specified in Technicon information Bulletin N 21a[4] .

Theory

All factors which were considered possible variables are tabulated thus:

1) Variations in sampling time and consequent errors in sample volume.
2) Pump surges, resulting in:
   a) Variable sample reagent mixtures.
   b) Pressure variations in the pump lines.
3) Errors introduced during dialysis, caused by back pressure or variable membrane permeability.
4) Electronic instability of the photometer or recorder.
5) Variations in air temperature and intensity of extraneous light.
6) Fluctuation in the extract velocity of the burnt gases.
7) Variable volumes due to manifold elasticity and carry-over from the previous samples.
8) Incorrect or fluctuating gas pressure.
9) Inconsistent thermocompensation, coupled with wrong neutral density filter combinations.
10) Method of washing between samples, and poor bubble patterns.

Since the errors applied particularly to sodium estimation, the effect of all variables on this constituent were considered, rather than for potassium. Fluctuations of standard values for potassium were less significant in view of the relatively large pen movement, for a given unit of potassium concentration.

Method

The result of each day's analysis showed no specific trend, thus suggesting that the cause of variation was either:—

a) A combination of several or all of the above factors, which in some permutations were magnified or decreased.
b) One random factor, the magnitude of which was entirely variable.

Table I shows the results obtained over a period of one week for standards placed at every seventh position of the sampler tray. Reference to this table shows that even if one allows $\pm$ 2 standard deviations, at least one result for each day is outside this range, indicating a random, fluctuating variable.

1) Sampling Errors

Since the first possible source of error was considered to be the sampler, a series of consecutive samplings was timed and the results tabulated. In order to obviate as much as possible any further errors, the cups were all filled to the same height and the

Table I

Day	Standard	Variation from known value	± 2 Standard Deviations
1	1	+ 1	3.84 M. Eq.
	2	0	
	3	+ 6	
	4	+ 5	
	5	0	
	6	+ 2	
	7	+ 5	
2	1	− 1	2.76 M. Eq.
	2	− 4	
	3	− 1	
	4	− 4	
	5	− 2	
	6	− 2	
	7	+ 1	
3	1	− 2	2.9 M. Eq.
	2	+ 2	
	3	+ 5	
	4	+ 1	
	5	+ 3	
	6	+ 4	
	7	+ 1	
4	1	+ 2	2.3 M. Eq.
	2	+ 1	
	3	0	
	4	+ 3	
	5	+ 3	
	6	+ 1	
	7	+ 4	
5	1	− 4	2.36 M. Eq.
	2	− 3	
	3	− 1	
	4	0	
	5	+ 1	
	6	+ 2	
	7	− 1	

time taken from the instant the tube touched the liquid, to the instant it left the surface. Table II shows the results of this observation, from which the arithmetical mean of the time was calculated as 59 seconds. For a delivery tube sampling 0.32ml/min. this time represents a volume of 0.314 ml.

However, from the table the sampling time can be seen to vary by a maximum of one second, which corresponds to 5.3×10^{-3} ml. of sample. If this is expressed as a percentage of the mean volume an error of ± 1.7% is obtained. With this figure in mind, a hypo-

TABLE II

No. of Sample	Time	Deviation from mean (59)
1	59.5	+ 0.5
2	60.0	+ 1.0
3	60.0	+ 1.0
4	60.0	+ 1.0
5	59.0	—
6	60.0	+ 1.0
7	59.5	+ 0.5
8	59.5	+ 0.5
9	59.5	+ 0.5
10	59,0	—
11	59,0	—
12	59,0	—
13	59,0	—
14	58.0	— 1.0
15	58.5	— 0.5
16	58.5	— 0.5
17	59.0	—
18	58.0	— 1.0
19	58.0	— 1.0
20	59.0	—
21	58.5	— 0.5
22	59.0	—
23	59.0	—
24	59.0	—
25	59.0	—
26	59.0	—
27	59.5	+ 0.5
28	58.0	— 1.0
29	59.0	—
30	59.0	—

thetical error for the final result can easily be deduced by the following reasoning:—

a) Irrespective of any variation in sample volume, the volume of diluent (ml./min.) can be taken as constant, since this depends only on the pump rate.

b) Assuming that the percentage of the diluted sample actually dialysed remains constant, a total sample volume of 0.314 ml., followed by one of $0.314 \pm 5.3 \times 10^{-3}$ ml., will result in considerably different dilutions. For a mean sodium value of 150 M.eq. an error of $\pm$ 2.5 M.eq. can thus be obtained.

It may be suggested however, that the actual volume of sample is not critical, since the percentage dialysed does remain constant. Thus, it is only necessary to ensure that the sample-recipient stream flows through the photometer just long enough for the pen to record the maximum concentration.

To test the validity of this reasoning, the sample tube was deliberately withdrawn from the specimen before the time sequence was completed and the resulting graphs compared. This showed that for a 60 second sampling time, up to 3 seconds difference could be tolerated without any gross change in reproducibility, but

for longer periods the peaks fell off rapidly. Whilst this finding may appear to detract from the significance of the one second maximum sampling error, the very fact that exact duplication of conditions is not being achieved must surely be regarded as detrimental to a continuous flow system.

2) Pumping Errors

Since the fluid stream was aspirated directly into the flame from the dialyser, it was thought that pump surges could be a source of error, since such a phenomenon would result in an unstable flame. Accordingly the dialysate was collected and aspirated directly into the flame, using the suction of the gas flow and air pressure as motive force.

To eliminate hydrostatic pressure variation as much as possible the sample was aspirated from a large petri dish. This resulted in a considerably more stable base-line, thereby lending strong suspicion to pressure variation as a source of error.

3) Dialysis Errors

As a result of the above findings, the possibility of further pressure changes occuring during dialysis was investigated. A dilution of the 160 M.eq. standard was made in lithium sulphate and water, and pumped directly into the flame on continuous aspiration. This resulted in a rather ragged baseline and, when sampled as for consecutive tests, variations of up to 4% transmission were obtained. While these findings supported pressure variation errors, they also indicated that the smoothing effect of the dialyser was necessary for the continuity of the baseline.

With regard to variable membrane permeability, it was noted that mention was made of acid conditioning of the membranes in *Technicon Photometer Fault Finding Bulletin* (1965). To find the magnitude of this effect, if any, several commercial control sera were dialysed on continuous aspiration and the recipient stream collected, after first allowing five minutes flow to waste in order to achieve a steady state. The sodium content of the dialysate was then estimated manually, using an Evans Electroselenium Limited (E.E.L.), flame photometer. By comparing the observed value for the dialysed sera with that obtained for a known 1 in 50 dilution, the per cent. dialysed was calculable. This value was then multiplied by the dilution figure (in this case 1 in 10.6) to give the actual per cent. dialysis. The results obtained for five different sera are shown in Table III.

Reference to table III shows that sample four varies from the mean per cent. dialysis of 26.91% by +0.28%, which is approximately +1.7 M.eq. for a 150 M.eq sample. It may reasonably be argued that the limitations of the E.E.L. instrument and the involved experimental error do not allow calculation to three, or even two, decimal places, but the above results do show that there is a high degree of probability in dialysis being a source of error.

TABLE III

Sample	Calculated Dilution	% Dialysis	Actual % dialysis x 10.6 for dilution
1	1.2615 in 50	2.523	26.74
2	1.274 in 50	2.548	27.02
3	1.268 in 50	2.536	26.87
4	1.282 in 50	2.564	27.19
5	1.263 in 50	2.526	26.77

4) **Electronic Instability**

Instability of the electronic circuits was next considered and since visual inspection showed no obvious defects, the whole photometer unit was sent to an electronics engineer for complete overhaul. The valve complement of the recorder was also replaced, but subsequent analyses showed little improvement.

5) **Extraneous Light and Temperature Effect**

Room temperature and humidity was considered a likely cause of electronic variance, resulting in inconsistent standards. This would be expected particularly on days when sunlight was shining directly on to the photometer control panel, heating the instrument and, perhaps more importantly, introducing extraneous light of variable intensity.

To eliminate this possibility, the extract chimney was made adjustable, so that a close-fitting connection could be made with the photometer chimney, whilst the sunlight was dispersed with Venetian blinds. A thermometer attached to the control panel showed a variation of only 2°C. over a one hour period with no obvious correlation to standard variation, which again showed no improvement.

6) **Gas Extraction**

Fluctuation in the velocity of extraction of the burnt gases, whilst not in itself a likely cause of error, would invariably result in flickering of the flame; a most undesirable occurrence. It was found, however, that with the chimney right down on the photometer to exclnde light, the instrument heated up considerably and resulted in a marked shift of the baseline. This effect was eliminated when the extract chimney was left raised about eighteen inches, as presumably were any causes of the flickering.

7) **Manifold and Carry-over Errors**

Manifold elasticity resulting in variable tube diameters was next considered and, accordingly, sample, diluent and recipient pump tubes were changed every three days for a period, but this did not result in any noticeable improvements.

As with all continuous-flow systems the effect of sample carry-over must also be considered as a potential source of error. To find the magnitude of this effect a series of 150 M.eq./l. standards were aspirated, every third sample being replaced by a 120 M.eq./l.

standard. While a variation of 30 M.eq. would seldom be encountered in clinical practice, the percentage carry-over and subsequent numerical value should be applicable to any successive samples which vary by more than 10 M.eq.

The mean value obtained for ten 120 M.eq./l. standards for this test was 121.4 M.eq./l. Substituting the above figures in an expression formulated by Allan (1966)[1].

i.e. $$\Delta = \frac{C_1 - C_2}{C_3 - C_1}$$

gave a value of 5% for Δ where, Δ = fraction carried over.
C_1 = apparent concentration.
C_2 = true concentration.
C_3 = value of previous specimen.

Once Δ has been found correction can be made for samples by substituting in equation derived from the above as;

$$C_2 = C_1 + \Delta C_1 - \Delta C_3$$

which, in this case gave a value of 119.5 M.eq., for the true concentration. Because this value is slightly lower than the known value, the amount of compensation for carry-over (the value of Δ) is too large. This in turn shows that sample carry-over, whilst in itself a cause of error, is coincident with some, or all of the variables so far demonstrated.

8) Gas Pressure

Incorrect or fluctuating gas pressure is a well recognised source of error in flame photometry and, to eliminate this possibility, all gas gauges and regulators were stripped and cleaned. A new burner unit and mixing chamber were also fitted.

Whilst the former produced little improvement, the new burner unit resulted in a considerable gain in reproducibility.

9) Thermocompensation

Thermocompensation was next considered and to ascertain the degree of drift the photometer was left running on continuous aspiration of the 160 M.eq. standard for a period of 90 minutes. The resulting baseline was found to be quite steady with a maximum variation of $\pm$ 0.5% transmission, but had a slight drift towards zero. Standards aspirated after a short equilibration time showed a marked trend towards zero, which was easily compensated for by thermistor adjustment. These findings, whilst perhaps of significance, do not explain erratic variation.

10) Inter-sample Wash

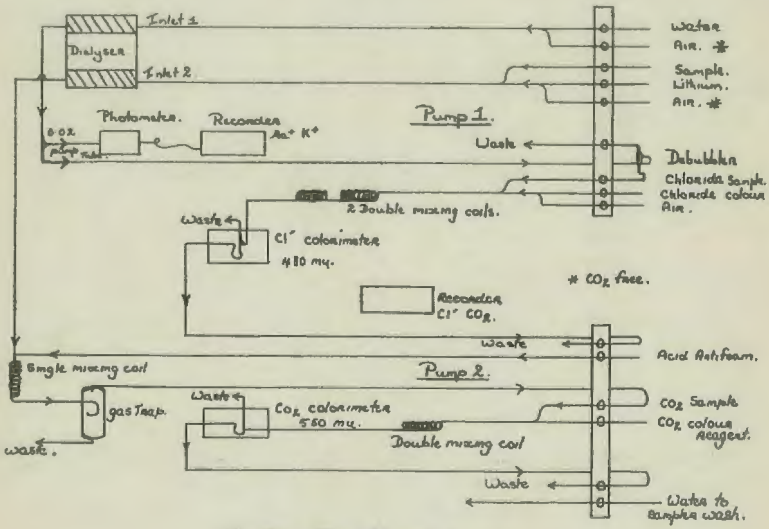
It was found that the peaks tended to drop if the water wash was omitted, thus a pump tube was incorporated to feed the reservoir, as specified in Technicon Manifold N 21a.

The importance of a steady bubble pattern is also emphasised, as small erratic bubbles may coalesce and manifest as a sudden jump on the recorder chart.

Results

On the basis of the above findings it would appear that the investigated variables were indeed potential sources of error, but there is little that can be done to eliminate them as they are an integral part of the system. However it was felt that the effects of pressure variation could be diminished by modification of the original manifold, as shown in Fig.2. Since the line from the dialyser to the photometer returns to pump 1, it may be assumed that any variations in speed or platten pressure between the two pumps are thus eliminated as a source of pressure differences.

The diluent and recipient pulse suppressors were also removed, resulting in a more steady bubble pattern. Daily standards are now reproducible to ± 1.5 M.eq., although an occasional one may show greater variation.



Modified Manifold. Fig. 2

Discussion

Thiers and Oglesby (1964)[3] found one standard deviation for sodium to be 2.3 M.eq., whilst Campbell and Annan (1966)[2] calculated one S.D. at 1.5 M.eq., based on 563 determinations. In this case, although only 35 values were used, a figure of 1.4 M.eq. was obtained; a result comparable with that of the latter workers. It was also noted by Thiers and Oglesby (1964)[3] that gross variations occurred if the volumes in each cup varied, a fact which must be considered when standard cups are being used several times in each batch of analyses, for reasons of economy.

Campbell and Annan[2], have also shown that the standard deviation increases directly as the sample value becomes higher, indicating a decrease in sensitivity. This effect can be mimimised, however, if the greatest spread in terms of %T., is obtained for the standards. In this laboratory, after numerous neutral density filter combinations had been tried, a spread of 45% T. was obtained for the 120 — 160 M.eq./l. standards, thereby achieving maximum sensitivity over the clinical range.

Conclusion

The results of these experiments showed that: —

a) Some variation is unavoidable and, on the face of it, 100% reproducibility can never be attained, due to the limitations of the machine and extraneous factors.

b) Greater accuracy could be obtained by incorporating more standards as a drift control, and drawing a new line on the reader chart for each standard which varied by more than ± 1 M.eq. from the preceding standard. This practice is described in detail by Thiers and Oglesby[3] and appears to be the only way to overcome the problem at the present moment.

c) Despite these limitations, semi-automation has resulted in improved reproducibility in the clinical laboratory (a factor of prime importance to the clinician) and has certainly eliminated gross errors associated with manual techniques.

Acknowledgments

I am most grateful to Mr R. D. Allan, Chief Technologist, for his technical advice and encouragement in preparing this article and to Miss E. Pearce, for her assistance and tolerance of my disruptions to the daily schedule.

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— TILL DEATH US DO PART —

or until we retire, the N.Z.I.M.L.T. Conference will be a major event of the year.

PLAN — PREPARE — PRODUCE

Papers of 10 minutes duration (5 minutes if you are shy) will be needed at the Napier Conference.

The gavel will be strictly and unceremoniously applied, but you will receive your allotted time.

The Eradication of Diphtheria as a Major Infectious Disease

MARILYN GLEN-KILLEEN
Pathology Department, Green Lane Hospital, Auckland.

Winner in the Essay Section of the Junior Essay Competition, 1967.

The disease diphtheria was first observed and recorded as a clinical syndrome by Bretonneau in 1826. Loeffler, in 1884, proved that the disease was bacterial in origin and that it was caused by a single organism, the diphtheria bacillus, which is now known as *Corynebacterium diphtheriae*. He demonstrated the bacillus in the throats of thirteen out of twenty-two diphtheria patients, and isolated it from six of them in pure culture. Subsequent inoculation of guinea-pigs with the bacillus caused death in two to five days, and at autopsy the animals showed localised haemorrhagic oedema, blood-stained pleural effusion, pulmonary consolidation and congestion of the adrenals. A significant point noted was that the bacilli could be isolated only from the site of inoculation, and not from the affected organs. Roux and Yersin, in 1888, discovered that death was due to the presence of a potent extracellular toxin and not to the dissemination of the bacillus through the body. It is now believed that the action of the toxin on the heart muscle is one of the chief causes of death in diphtheria.

The typical symptoms of diphtheria are: fever, malaise, headache and nausea. In most cases there is also a slight swelling and redness of the tonsils, possibly with the formation of a false membrane. There have been three different strains of the diphtheria bacillus identified — gravis, intermedius and mitis. In general the gravis and intermedius types are the predominant ones, causing a more severe form of the disease, with a high case-fatality rate. As the disease is essentially a toxaemia, evidence suggests that the gravis and intermedius strains are able to multiply more rapidly within the body, and therefore produce more toxin. There is, as yet, no proof of any qualitative difference in the nature of the toxins produced by the three types.

Diphtheria is spread mainly by personal contact, and is typically a disease of households, schools and institutions, where children are in close contact at susceptible ages. Evidence appears to be against the view that fomites play any significant role in its spread. The bacillus can be transmitted by those suffering from the disease, by healthy carriers and by those who are recovering from it but who are still retaining virulent bacilli in their throats.

The first step in the eradication of diphtheria occurred in 1913, when von Behring reported the successful use of a toxin-antitoxin mixture for the prophylactic immunisation of children.

The use of such mixtures became quite popular, but they were, however, rather dangerous. An improvement was made with the discovery that the treatment of the toxin with formalin deprived it of its toxicity, while leaving its antigenic power unaltered. Further treatment of this formol toxoid, by precipitation with alum, formed an agent of high antigenic potency, which when properly used could give a fairly high degree of protection against diphtheria.

The age at which immunisation is performed should depend primarily on the age distribution of diphtheria in the country concerned. Before artificial immunisation was introduced, diphtheria was mainly a disease of small children. It occurred at an early age and resulted in widespread production of antitoxin in the population. A mild reinfection during adult life stimulated the maintenance of antitoxin levels, and thus most members of the population, except for children, were immune. The introduction of artificial active immunisation has changed this situation. After active immunisation during the first five years of life, antitoxin levels generally remain adequate until adolescence. Following either natural or artificial immunisation, antitoxin levels last only a limited period of time, and with the lack of carriers of the bacillus the stimulus of subclinical infections is lacking. Consequently, many adults and adolescents have no significant amount of antitoxin in their bloodstream.

The principal aim in preventing or eradicating the disease must therefore be to limit the distribution of toxigenic bacteria in the population, and to maintain as high a level of active immunisation as possible.

To determine the effect of immunisation, the Schick test may be employed. This test is based on the fact that diphtheria toxin is very irritating and results in marked local reaction when injected intradermally, unless it is neutralised by circulating antitoxin. A standard dose of toxin is injected into the skin of one forearm and an identical amount of control toxin into the other. Results are read at forty-eight hours, and redness and swelling indicate that antitoxin is absent from the bloodstream, and therefore there is no immunity.

A high Schick negative, or conversion rate of positive to negative, is a fairly good indication of satisfactory immunisation. The achievement of a high level of immunity depends a great deal on the quantity and quality of the prophylactic used, and also on the follow-up of the necessary "booster" doses. The ultimate proof of the effectiveness of immunisation is the behaviour of the treated persons when exposed to the risk of natural infection.

In England and Wales, between 1940 and 1943, 4,829,115 children under fifteen years of age were immunised. The estimated

child population under fifteen in 1943 was 8,583,000. Analysis of the 1943 returns showed:—

Annual Rates of Incidence per 1,000 child years

(a) Immunised 1.16

a : b = 1 : 3.5

(b) Non-immunised 4.06

Annual Rates of Death per 1,000 child years

(a) Immunised 0.0104

a : b = 1 : 25

(b) Non-immunised 0.260

This showed that the risk of contracting diphtheria was lowered three to four times by immunisation, and the risk of dying from it twenty times. Taylor, Tomlinson and Davies in London, in 1962, showed that immunisation lowered the risk of clinical diphtberia about six times, but only when performed during the previous five years.

It must be emphasised that protective inoculation does not confer absolute immunity. In the Detroit outbreak of 1956 over 75% of the affected kindergarten children, and 94% of the children at one school, had a history of inoculation in early infancy or during the pre-school years. Studies by Hartley and Tulloch on the outhreaks in Tyneside and Dundee respectively, showed that diphtheria can attack a fully inoculated person, although it seldom proves fatal. Once the antibody producing mechanism is sensitised, infection with the diphtheria bacillus stimulates rapid production of antitoxin, invariably protecting against death.

It is thought that the best way to eradicate diphtheria completely is to raise the general level of immunity in the community above the critical level where the chain of infection is broken. To achieve this it appears that, in a fairly densely populated area, at least 70% of school and pre-school children must be immunised and kept immune by reinforcing injections.

Objections to this method have been raised on the grounds that it may lead to a rise in the proportion of healthy carriers. However, the fewer cases there are the fewer contact carriers there will be, and therefore the chances of non-immune subjects contracting diphtheria will be considerably lowered. This has been proved by experience. A survey, in 1950-51, of 21,000 children in Leeds, where immunisation had been carried out consistently for ten years, failed to show a single carrier of virulent bacilli.

In the general population, diphtheria appears to be well-controlled by prophylactic immunisation. This measure is based on well tested principles, is effective in raising resistance to the disease and gives a high protection against death.

If carried out on a wide enough scale, diphtheria inoculation could possibly lead to the complete disappearance of the disease.

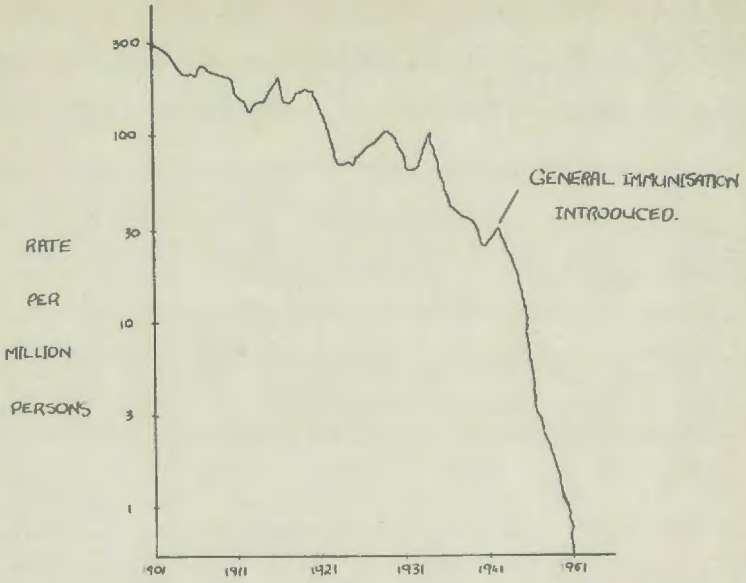

Figure 1. The Diphtheria Death Rate in England and Wales 1901-51, showing the precipitous fall in the death rate following the introduction of general immunisation.

Technical Communications

Staining Brucella Organisms

Sir,

The presence of Brucella organisms in direct smears can easily be overlooked when stained by the conventional Gram stain technique. This failure is largely due to the following:—

a) The presence of large amounts of extraneous Gram negative material in the smear, which tends to mask Brucella organisms.

b) The size of Brucella organisms and the fact that they are Gram negative.

As an alternative stain, Koster's modified Ziehl-Neelson (1941) is far superior to the conventional Gram stain. However, I have found a variation of Koster's method more suitable for routine laboratory work. It is performed as follows:—

Modified Koster's Method

Films are made and fixed in the usual manner.

1. Stain with dilute carbol fuchsin 3-5 minutes without heating (Ziehl-Neelson carbol fuchsin 1 part to 9 parts of distilled water).
2. Wash with tap water.
3. Decolorise with 0.5% acetic acid for 10 seconds.
4. Wash with tap water.
5. Counterstain with alkaline methylene blue for 20 seconds. (Saturated solution of methylene blue in alcohol 30 ml. KOH 0.01% in water 100 ml.)
6. Wash with tap water, blot dry and examine under oil immersion.

This modified Koster's stain relies on the principle that Brucella organisms are acid-fast when decolorised with weak acids. Hence when this stain is applied to direct smears the Brucella organisms stain red and all other organisms and extraneous material stain blue. The contrast is most marked and Brucella organism, if present, are obvious.

I have used this modified Koster's stain extensively in this laboratory and have obtained very satisfactory results. I feel sure that it has its application in a medical microbiology laboratory and wish other workers equal success.

R. PEARSON.
Department of Animal Health,
Massey University,
Palmerston North.
15 August, 1967.

REFERENCE

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Toxicological Investigations and the Laboratory

Sir,

It may seem surprising, with the ever-increasing use and abuse of pharmacological preparations, that little or no toxicological investigations are done in the routine clinical laboratory.

One reason for this may be the difficulty in differentiating forensic toxicology from analysis for purely clinical purposes.

Ideally, forensic examinations would be performed by the Government Analyst. He is an experienced witness and could, if necessary, call upon the full facilities of the Department of Scientific and Industrial Research.

However, at a purely clinical level, there is undoubtedly a need for rapid screening techniques to use in testing for acute poisoning.

In practice of course a sharp division is impossible. For instance, a blood alcohol determination may be performed on a patient with a head injury — in order to plan or assess treatment. But if the patient is suspected by the police of being a "drunken driver," the result of the blood alcohol analysis may become a forensic exhibit. It is not commonly realised that the police have the right to demand the result of any investigation done in the course of treating a patient. One answer to the quandary would be for the clinical laboratory to use simple (relatively crude and quick) techniques. The results, although being of clinical value, would not be acceptable in a court of law! An example of this *should be* the blood "alcohol" determination, using the acid-bichromate reduction method.

This time-honoured technique is *not* a specific method for determining ethanol, and as such cannot give unequivocal results. Since a defendant's guilt or innocence may depend on the result, should be specific technique not be mandatory? *e.g.* Gas chromatography or to a lesser extent the alcohol dehydrogenase-diphosphoropyridine nucleotide techniques. In fact, the acid-bichromate results are accepted in a court of law.

We recently had experience of a case where a simple toxicological test proved to be of clinical value. The patient, a middle-aged female, was admitted to hospital in a confused and disorientated condition. There was a long history of myocardial ischaemia and anaemia of unknown origin. The day prior to admission the patient was investigated by a private laboratory.

The venous blood obtained was chocolate brown in colour. One of the tests performed was for detecting a glucose-6-phosphate-dehydrogenase deficiency. This was negative. The hospital laboratory received some of this specimen for haemoglobin investigations. The plasma contained oxyhaemoglobin and meathaemalbumin. Since the specimen was anticoagulated with oxalate at least some of the oxyhaemoglobin was probably an artifact.

Surprisingly, in view of the colour of the blood, a whole blood haemolysate did not reveal sulphaemoglobin or methaemoglobin. On the day after admission the patient collapsed, with what was

later found to be an acute haemolytic crisis. Some urine was obtained about this time and was cherry red in colour. Oxyhaemoglobin and methaemalbumin was found; methaemoglobin and sulphaemoglobin were not detected.

Since chocolate coloured blood is sometimes found in phenacetin poisoning[2] a simple screening test for this was performed.[3]

The Test

To 1 ml. urine add 2-3 drops diluted hydrocholoric acid. Cool in ice. Add 2-3 drops freshly prepared 1% (w/v) sodium nitrite solution, followed by 2-3 drops *a*-naphthol reagent. *Reagent:* 100 mg. *a*-naphthol dissolved in 5 ml. 2 normal sodium hydroxide solution. Prepare fresh. Negative and positive (para-aminophenol) controls were also performed. In spite of the intrinsic colour of the urine in question, the test gave a clear positive result — a red colour, due to excretion of para-aminophenol, derived from the ingestion of phenacetin.

It was found that four days prior to admission to hospital, the patient had obtained 100 A.P. Codein tablets. She consumed all of these over the four days. The patient died on the second day after admission to hospital. Histological examination of tissue taken at autopsy revealed a analgesic nephropathy. The medical literature abounds with references to the results of analgesic abuse, and in particular its implication in chronic interstitial nephritis.[1,5]

The consequences of the notorious Hjorton's powder (named after the general practitioner who formulated the mixture) was described in 1963 by Grimlund.[4] This preparation was prescribed during the 1920 influenza epidemic in a small village in Sweden. It consisted of caffeine, phenacetin, and phenazone. Unfortunately its use outlived the epidemic and, to quote Grimlund, " In every life it became almost as common to offer somebody a 'powder' as a cigarette."

When the inordinately high number of deaths due to renal disease were associated with Hjorton's powder, the formula was modified by omitting the phenacetin and phenazone!

A deficiency of glucose-6-phosphate-dehydrogenase causes an increased susceptibility to haemolysis following the ingestion of phenacetin. But haemolysis is known to result in patients without this defect.[6]

J. L. BRAIDWOOD.
Dunedin Hospital.
28 August, 1967.

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Scientists at the Pasteur Institute Discover that a Vibrio Purifies Polluted Waters

FERNAND LOT

It had been observed for some time that rivers become amazingly clean downstream from the big cities. At Clichy for instance, at the mouth of the main Paris sewer, the number of bacteria present in one cubic centimetre of water amounts to some three million, whereas about forty kilometres downstream (25 miles) the Seine is so clear of organic matter and most of its microbes that it has no more than when it entered Paris.

Up till now, action by oxygen and sunlight was held to account for the somewhat rapid spontaneous cleansing. In actual fact the process is quite different, as has recently been discovered. The importance of this discovery has not escaped the attention of members of the *Academie des Sciences* who received a note by Pierre Lepine of the Academy and Drs Antonina Guelin, Jacqueline Sisman and Daniele Lamblin entitled "Electron microscope study of *Salmonella* lysis by *Bdellovibrio bacterioborus.*" The research workers at the Pasteur Institute obtained a most unexpected result, probably rich in its consequences, thanks to judicious placing as it were of question marks.

At the start, on closer examination, it was obvious that neither ultraviolet rays (which do not go further than half a millimetre into the water) not oxygenation (the asepticising effect of which is a slow process) were enough to cause such prompt and generalised action on the thick culture medium that a river is when it flows out of a large city. It was then thought that the bacteriophage, feeding on bacteria as the name indicates, and which is a virus, must be involved.

An attempt was made to find out and in the Bacteriophage Department directed by the son of Charles Nicolle, Dr Pierre Nicolle, Dr Antonina Guelin and he carried out a vast and exhaustive survey, examining the microflora of all the waters in the world, rivers ranging from the Ganges to the Mississippi.

All data and investigations pointed to rejection of the hypothesis. Bacteriophage, which also gave disappointing results when brought in to destroy pathogenic germs inside the organism, was absolutely inoperative in the case of the natural purification of waters. (It should be noted however that it has become a marvellous instrument for identifying different types of bacteria according as to whether they are subject to lysis—dissolving, destroying—or not if acted on by a given type of bacteriophage; hence the *lysotype* method and its useful applications on which Pierre Nicolle is working. Lysotyping has already made it possible to change the position in the epidemiological fight against typhoid fever and other infectious diseases.)

Another secret agent had to be found.

In 1963, two German bacteriologists, Stolp and Starr, specialists in soil microbes, isolated from earth and described a minute vibrio they called *Bdellovibrio* and qualified as *bacteriovorus,* since it proved able to penetrate "Gram negative" bacteria and destroy them. Perhaps this was the determining factor in the biological decontamination of waters? It did prove that this microorganism did indeed produce that happy result.

With warm encouragement from Professor Lepine, Dr Guelin embarked upon a further series of investigations. She looked for this vibrio everywhere and used the electron microscope to follow the different phases in the destruction of bacteria attacked, in particular the evolution of lysis in typhoid bacilli.

A first observation: wherever the water examined came from, whenever it was polluted—whether from the Seine, the Rhine, the Ganges, the Baie des Anges or Pacific Islands—*Bdellovibrio* was found. (All naturally pure water such as spring water, artesian well water, is without.) And that is why polluted water—sewer, river and sea water, have an amazing bactericidal power. At the start of this research in 1966, one hundred thousand vibrio were found per cubic centimetre in Paris sewer water. If a suspension of a billion germs be put in a test tube, it becomes clear in about forty-eight hours, as a result of the introduction of a few *Bdellovibrio.* If this suspension be autoclaved, it remains turbid, which shows that a direct biological attack has indeed taken place.

What does the electron microscope reveal? The striking photographs taken by Dr Jacqueline Sisman and enlarged forty thousand times, show the whole process. Whenever a *Bdellovibrio* touches the wall of a bacterium, it becomes permeable to water and the bacteria tends to burst from difference in osmotic pressure. The *Bdellovibrio* always penetrates the bacterium through a depression in the membrane, with its end that has no flagellum. A sort of swelling of the bacterium is then observed; its diameter increases considerably and it looks like a blown up bag, while the protoplasm shrinks, pales and is soon filling only half the membrane. During this time, inside the bacteria itself, multiplication of the intruders occurs. Division takes place by elongation and binary segmentation, the *Bdellovibrio* therefore generally splitting in two and the segments grouping in pairs within the bacteria. After thirty-four hours this is completely filled with *Bdellovibrio* which finally escape from the dislocated bag—and hasten to parasitise other bacteria which will inevitably be destroyed in their turn. By the fifty-eighth hour there is nothing but free *Bdellovibrio* left in the preparation along with the remains of bacterial membrances: not a single bacterium left intact.

This should be put to very good use. Unlike bacteriophage, *Bdellovibrio* is not bound by certain restrictive conditions for multiplication. It can proliferate anywhere. It is harmless. Lastly, it does not seem to be specific, which means that it attacks various species of bacteria, not just one. It may well be possible to use it

for therapeutic purposes if appropriately introduced into an organism exposed to infection. It might also be called upon to speed up the water purifying cycle as a result of systematic seeding.

All this does more than elucidate a mechanism that had hitherto remained a mystery. It also opens up interesting prospects.

[A previously unpublished article supplied through the courtesy of the French Embassy.]

Book Reviews

Basic Electronics for Biologists. G. C, Ware, M.A., Ph.D. Paperback. 206 pages, 161 illustrations. J. and A. Churchill, London, 1967. Price in U.K. 30s.

The first fourteen chapters of this book set out to give a simplified account of basic electronics, and apart from devoting too much space to relays (three chapters), achieve a good balance between practical information and some understanding of circuit theory. Biological workers will, however, be disappointed to find no mention of the cathode follower or the differential amplifier.

The sections dealing with semiconductors lack some of the information necessary to construct reliable working circuits. Practical aspects of providing a heat sink for a transistor and, in the case of the transistor switch, the saturated mode of operation, are two important points not covered.

It was interesting to find an elementary book going to considerable lengths to cover the cathode ray oscilloscope, with examples of its practical application as a measuring device. A newcomer to the electronic field would have benefited if a discussion on the operation of a modern oscilloscope had been added.

The circuit examples, particularly in the later sections of the book, are not always directly relevant to biological work. If the book had treated subjects such as bridge circuits, recorders, pH meters, electrodes, electrophoresis, and counters at an elementary level, it would have given it a better orientation towards the biological field. Nevertheless, this is an easily read text I can recommend to biologists wanting an elementary and practical introduction to basic electronics. A.T.W.

An Introduction to the Haemoglobinopathies and the Methods used for their Recognition. H. E. Hutchison, M.D., F.R.C.P. (Glasg.), F.C. Path. Edward Arnold, London. 1967. 88 pages. Price in U.K. 30s.

The recent advances in the knowledge of the normal structure of haemoglobin and the inherited abnormalities of the molecule have opened up another new and complex field in haematology. The author of this small book has presented an up-to-date, brief but comprehensive account of the haemoglobinopathies which should be of great interest to both medical and technical workers in the haematology laboratory.

Part one of the book deals firstly with the structure of the types of haemoglobin normally found and then discusses how abnormalities arise, their nomenclature and geographical distribution. The last three chapters in this section cover the clinical aspects, inheritance, haematological findings and the mechanisms of the anaemia which may occur.

Part two, which occupies the remaining twenty-four pages, deals with the methods used in the recognition of the haemoglobinopathies. The tests which the author suggests should be used in the preliminary investigation are the osmotic fragility, sickling test, supravital staining for erythrocyte

inclusions, alkali denaturation (with examination of cellular distribution of HbF if increased), and electrophoresis. All but the first of these methods are dealt with in detail, each one being introduced with the principle of the test. Starch gel electrophoresis is discussed in great detail, paper and agar gel are briefly mentioned but unfortunately, no reference is made of the use of cellulose acetate. This last medium is probably more convenient for many laboratories to use, as it will already be used for the electrophoresis of serum proteins. Three simple tests to detect the presence of unstable haemoglobins are also given.

The final chapter covers some of the more complex techniques such as hybridisation and finger printing, which may be used in the further characterisation of haemoglobin variants.

Although the author states that his book is intended for the medically qualified laboratory worker, it should also prove to be very useful for the medical laboratory technologist. The cost of this volume, however, does not compare favourably with some of the more comprehensive works on this subject. A.D.N.

Microbiological Methods. (2nd Edition), C. H. Collins, M. I. Biol., F.I.M.L.T. 404 pages, numerous illustrations. Butterworth, London. 1967. Price in U.K. 62s.

This textbook is one of the range in the Laboratory Techniques Series and was first published in 1964.

The first of the four sections into which the text is divided deals with the general biology of micro-organisms, classification and serology, and although covered in forty pages serves as a useful introduction to microbiology.

The second section covers apparatus, sterilisation, preparation and cleaning of glassware. The chapters and illustrations on thermostatic control, preparation and testing of culture media and the estimation of bacterial numbers provide much valuable information. Included in this section is a chapter on complement fixation by C. E. D. Taylor.

The third section is devoted to methods for the identification of the main groups of micro-organisms and includes numerous tables and tests for their differentiation.

The final section, on applied microbiology, includes methods for testing water supplies, milk and milk products, foods, kitchen hygiene, antibiotic assay, testing of disinfectants and concludes with a short section on safety in the microbiological laboratory.

The author states in the preface that this book is almost entirely practical in its scope and is intended to be used as a "bench book" to supplement the more academic and theoretical textbooks. The author has succeeded in his intention and this book should prove of value in the routine laboratory. J.T.H.

Progress in the Chemistry of Fats and Other Lipids. Volume X, Part 1. **Fatty Livers and Lipotropic Phenomena.** Colin C. Lucas and Jessie H. Ridout. Editor, Ralph T. Holman. Pergamon Press, Oxford. 1967. 149 pages. Price in U.K. 50s 0d.

This volume gives a detailed account of the occurrence and metabolism of lipids in normal and fatty livers. Most of the work referred to is of an experimental nature using rats and other animals, though adequate mention is made of the fatty livers occurring in humans. The manipulation of liver fat by diet is very fully reviewed as are the biochemical mechanisms underlying these changes.

The discussion on the extraction of liver lipids would be useful to anyone using extraction techniques.

Hospital technologists would be interested in the comparison of results of lipid determination in livers by histological staining and by chemical extraction. The results of the two methods are not always in agreement.

This is a concise but thorough review (1,189 references) of the nutritional and biochemical disturbances causing fatty livers. G.G.D.

Virus and Rickettsial Diseases of Man. Fourth Edition. Sir Samuel Bedson. F.R.S., M.D., D.Sc., F.R.C.P.; A. W. Downie, F.R.S., D.Sc., M.D., F.C. Path.; F. O. MacCallum, B.Sc., M.D., M.R.C.P., F.C. Path.; C. H. Stuart-Harris, C.B.E., M.D., F.R.C.P. 462 pages. Edward Arnold, London. 1967. Price in U.K. 90s 0d.

The previous edition (3rd) of this work was published in 1961. The present edition (4th) attempts to incorporate the numerous advances in medical virology during the last 6 years. In particular the relative importance of viruses, rickettsiae, bedsoniae and mycoplasmas causing diseases of the respiratory tract are discussed. There is also a new, though unexciting, chapter dealing with tumour viruses.

The subject matter is divided into 30 chapters. The first four deal with general properties of viruses, epidemiology, immunity and recovery, and the diagnosis and treatment of viral and rickettsial infection. The following 22 chapters are concerned with particular virus-viral group infections and the clinical syndromes involving the nervous system, respiratory and gut tracts. Most of these chapters are presented under the sub-headings of: clinical features, pathogenesis and pathology, aetiology, characters of the virus, laboratory diagnosis, epidemiology, control-treatment. The four concluding topics include: animal viruses causing minor infections in man, viruses and tumours, diseases caused by bedsoniae, and the rickettsial diseases. References are given at the end of each chapter. A few of those cited are from 1966 and 1967 papers. Over 150 references support each of the chapters on rickettsiae, bedsoniae and acute respiratory diseases.

The format of this book is rather unappealing, it contains remarkably few illustrations or diagrams. All illustrations are in black and white. Ten quite useful tables are presented. One of these is entitled — Laboratory Diagnosis of Virus Infections in Man — and provides information as to what tests are used, how long the tests take to complete and the material required for investigation. Regrettably this table omits to mention respiratory syncytial virus, the parainfluenza viruses and the rhinoviruses. The text is thoroughly sound. The authors have been careful to point out the limits of present day knowledge. Theories and speculations are given no quarter. In the chapter on diagnosis no space was given to the three relatively recent techniques of electron microscopy, organ culture and immunofluorescence which promise not only to reduce the time required for diagnosis but also to demonstrate the more fastidious pathogens.

Virus and Rickettsial Diseases of Man is an accurate though conservative publication. It would appeal especially to physicians interested in infectious disease and to medical virologists. P.J.M.

Books Received

Laboratory Medicine-Hematology. Third Edition. J. B. Miale, M.D. 1,257 pages, 555 illustrations and 25 plates, including 10 in colour. C. V. Moshy Company, St Louis. 1967. Price in U.S. $21.00.

Selected Abstracts

Contributors to this issue: R. D. Allan, J. Case, J. Hannan, D. Tingle.

BLOOD BANKING

Routine Prenatal Screening for Atypical Antibodies. Allen, S. T., Dubner, M. S. and Mockler, N. D. (1967), *Amer. J. Obstet. Gynec.*, 99, 274.

1,810 prenatal patients were screened for atypical antibodies using pooled reagent cells. 41 isoimmunised patients were identified and 19 gave birth to infants affected with haemolytic disease.

Sensitised Rh-negative patients accounted for 1.4% of the group studied, while sensitised Rh-positive patients represented 0.9%.

63% of the antibodies detected were the results of sensitisation to factors other than D.

The value of screening all sera for antibodies regardless of past history, parity or Rh type is emphasised. .

Prenatal T-Transformation? A Case of Polyagglutinable Cord Blood Erythrocytes. Jorgensen, J. R. (1967), *Vox Sang. (Basel)*, 13, 225.

This is an account of a case of transient polyagglutinability of the red cells occurring in a newborn infant. The polyagglutinability is interpreted as T-transformation occurring in utero. The infant was suffering from neonatal jaundice but it was not considered that this was associated with the polyagglutinability.

CHEMICAL PATHOLOGY

Usefulness of a Simple Serum Beta-Lipoprotein Assay: An Epidemiological Study of an Active Montreal Population. Allard, C. and Goulet, C. (1967), *Canad. med. Ass. J.*, 97, 1,321.

In the course of this study in which various biological parameters were investigated, the determination of β-lipoproteins by a simple and quick immunochemical method was included to verify its applicability in clinical laboratories, especially those in which facilities for blood lipid determinations are inadequate, or in field studies. The possibilities of using this test to estimate total serum cholesterol and triglycerides were studied.

The β-lipoproteins were determined by a method which has been patented by Hyland Laboratories as the β-L test. Using the material as available on the market, one drop of the subject's serum is mixed with two drops of anti-human β-lipoprotein precipitin serum. A capillary tube (75 mm. in length) is then filled to a height of 60 mm. and sealed, then centrifuged for 5 minutes in a micro-capillary centrifuge. The length of the precipitate is then read to the nearest 0.1 mm. A value of 2.4 mm. or greater was verified to be a valid predictor of hyperlipoproteinaemia, *(i.e.,* cholesterol $>$ 250 mg. per 100 ml. and/or triglyceride $>$ 150 mg. per 100 ml.)

The β-L test is suggested as a very useful and reliable test in clinical practice for screening rapidly and inexpensively for hyperlipoproteinaemia. J.H.

Spectrophotometric Examination of Amniotic Fluid as a Means of Predicting Severity of Intrauterine Erythroblastosis. Muller, P. F. (1967), *J. Indiana St. med. Ass.*, 60, 914.

The antibody titre is very unreliable as an index of the severity of haemolytic disease of the newborn for in many cases it rises in the presence of an Rh negative, unaffected, infant; on the other hand, it fails to rise in severely affected cases. A more accurate method of determining haemolytic disease and its severity was needed.

Observing the yellow discoloration of the amniotic fluid in affected cases. Bevis in 1952 and Liley in 1961 suggested a quantitative measurement of the decomposition products of haemoglobin in amniotic fluid by

utilising the 450 mμ peak in spectrophotometric examination of amniotic fluid. This has proved to be a much more accurate indicator of the severity of the disease and is now an accepted clinical procedure.

Amniotic fluid should be protected from light and refrigerated, but not frozen. It may be stored for several days. If the tap is bloody it may be centrifuged and utilised but an effort should be made to identify the blood as maternal or foetal. This is because foetal blood hemolyses much more rapidly than maternal and can distort the spectrophotometric curve, producing greater deviation at 415-420 mμ. This becomes especially confusing because the amniotic fluid with severely affected foetuses in metabolic acidosis produces this same distortion.

If a recording spectrophotometer is not available, manual readings may be made at 10 mμ intervals and recorded on semilogarithmic graph paper. In haemolytic disease a peak will occur at 450 mμ. A lesser peak may be found at 410 mμ. Deviation from linearity at 450 mμ (size of peak) is the significant figure. J.H.

Estimation of Trace Metals in Biological Material by Atomic Absorption Spectrophotometry. Piper, K. G. and Higgins, G. (1967) *Proc., Ass. clin. Biochem.* 4, 191.

Details of determinations for Ca, Mg, Pb, Cu, Zn. Normal ranges in serum and urine are given. Increased sensitivity by addition of butanol, by chelation and extraction, by using the Boling three-slot burnerhead and by a dual atomiser system are discussed. R.D.A.

The Flame Photometric Measurement of Serum Copper and its Use in the Assessment of Patients with Malignant Lymphoma. Hobbs, C. B., Jelliffe, A. M., and Warren, R. L. (1967) *Proc. Ass. clin. Biochem.* 4, 197.

Serum copper is low in Wilson's disease. High values can occur in many conditions including lymphosarcoma and Hodgkin's disease. Serum copper may rise before actual clinical signs appear. A flame spectrophotometer with a high resolution monochromator is used, but see previous reference. R.D.A.

Colorimetric Method for Rapid Determination of Serum Arginase. Mellerup, B. (1967) *Clin. Chem.* 13, 900.

This enzyme occurs in the urea cycle, the main source being the liver. Increased serum levels are thought to indicate liver disease with a high degree of specificity. Urea is removed by Sephadex and the enzymic formation of urea from arginine measured by Coulombe's diacetyl monoxime-thiosemi-carbazide. R.D.A.

Serum Creatine Kinase in the Early Diagnosis of Myocardial Infarction. Smith, A. F., (1967) *Proc. Ass. clin. Biochem.* 4, 219.

At 24 hours following infarction, all patients examined had abnormal C.P.K. levels. 95% had abnormal S.G.O.T. levels and 86% abnormal H.B.D. levels. C.P.K. begins to rise at 4-6hrs., S.G.O.T. an hour or so later. The main diagnostic advantage of C.P.K. is its extra sensitivity. This must be considered in relation to the time and expense of adding a further method to the routine. The author favours Calbiochem Calsuls. R.D.A.

Serum Creatine Phosphokinase: Evaluation of a Commercial Spectrophotometric Method. Hess, J. W., MacDonald, R. P., Natha, G. J. W. and Murdock, K. J. (1967) *Clin. Chem.*, 13, 994.

Calbiochem Calsuls were used in which the final measurement is the reduction of NADP. As more than 5 minutes may elapse before the reaction becomes linear. 8 minutes wait before making O.D. measurements is recommended. If the initial O.D. is greater than 0.350, make a dilution of the serum with water. It is suggested that the lower results found with haemolysed serum is due to the presence of adenylate kinase. R.D.A.

The Determination of Ammonia in Whole Blood by a Direct Colorimetric Method. McCullough, H. (1967), *Clin. chim. Acta.*, 17, 297.

Venous blood is immediately deproteinised. The supernatant can be stored at —4°c. for up to 72 hours. The Berthelot nitroprusside-hypochlorite colour reaction is employed. Lower blanks are obtained than when resin techniques are employed. R.D.A.

Note on Slot's Method for the Specific Determination of Creatinine. Grofnetter, D. Janosova, Z. and Cervinkova, I. (1967), *Clin. chim. Acta.*, 17, 493.

After applying the Jaffe reaction, (alkaline picrate) only true creatinine disappears on acidification. Measurements are made in alkaline and acidified samples. Optimum reagent concentrations are suggested.

R.D.A.

CYTOLOGY

The Presence of "Malignancy-Associated Changes" in the Monocytes of the Peripheral Blood of Cancer Patients. Mattson, Joan C. and von Haam, E. (1967), *Acta cytol.*, 11, 308.

Investigators have recently become interested in the existence of morphological changes in benign cells from patients with carcinoma. These changes were first described as "malignancy-associated changes" (MAC) by Nieburgs *et al.* in 1959. The recognition of MAC in non-malignant cells from cancer patients could be a useful tool in cancer detection if a high degree of accuracy could be obtained. The ease with which peripheral blood films can be obtained make them an attractive choice of material for screening.

A double-blind study was established by the authors to test the consistency of nuclear changes in the monocytes of cancer patients. No heparinised or oxalated blood was used; all films were stained with Wright's. Slides were read with oil-immersion and a 10X ocular. A differential count of 400 cells was performed on each slide. Every monocyte counted was evaluated for the presence of the four nuclear changes described as follows by Chomet *et al.*

1. Coarse irregular chromatin clumps.
2. Irregularly prominent nuclear membrane.
3. Multiple regular areas of chromatin clearing with central pinpoint chromatin condensation.
4. Nuclear lobulation, folding and layering.

A slide was considered positive when more than 50% of the monocytes examined showed two or more of the four changes. Slides with an equal number of "positive" and "negative" monocytes were classified as suspicious.

The authors concluded that no correlation existed between the presence of malignancy and MAC in the monocytes in the 200 cases studied. The changes described by Chomet *et al.* were found not only in cancer patients but also in a large number of control cases, and the slightly increased frequency with which these changes occurred in cancer patients must be considered statistically invalid. J.H.

Malignancy Associated Changes (MAC) in Blood and Bone Marrow Cells of Patients with Malignant Tumours. Nieburgs, H. E., Goldberg, A. F., Bertini, B., Silagi, J., Pacheco, B. and Reisman, B. A. (1967), *Acta cytol.*, 11, 415.

A study of 171 blood films from patients with benign diseases and cancer revealed MAC in polymorphonuclear leucocytes and/or lymphocytes in 88.7% of patients with carcinoma, in 64.7% of those with lymphosarcoma and in 64.3% of patients with multiple myeloma. Of 87 patients without evidence of tumour, MAC were absent in 85.1% of the cases.

In cases in which the clinical, radiological and histological findings may not permit an unequivocal diagnosis of malignant tumour growth, the recognition of MAC is of significant value.

MAC in enlarged nuclei of polymorphonuclear leucocytes, lymphocytes, monocytes and megakaryocytes consisted either of numerous clear spherical areas surrounded by deeply stained chromatin bands or of an atypical disorderly nuclear structure.

For the study of MAC in routine blood films, preparations were made from finger tip blood and stained by the Feulgen method as well as with Wright-Giemsa.

The examination of normal polymorphonuclear leucocytes revealed a prominent chromocentre [a more solidly stained area] in each lobule. This chromocentre had delicate chromatin band attachments which extended to a moderate number of small chromocentres at the periphery of the lobule. In polymorphonuclear leucocytes with MAC this chromocentre was absent either in several or all lobules. The nuclear lobules of these leucocytes contained in place of the usually single prominent chromocentre either a characteristic orderly structure or a disorderly alteration. In the orderly nuclear alteration the MAC consisted of numerous clear spherical areas each surrounded by a rim of increased chromatin, the nuclear membrane having often the appearance of discontinuity in one or several areas. In the disorderly type of nuclear MAC the chromatin structure was in disorderly arrangement. The chromocentres seemed less numerous than in the orderly MAC but were deeply stained, enlarged and varied in size and shape. Occasionally a small number of clear spherical areas with an increased rim of chromatin were also present in these cells.

The clear spherical zones of uniform size and the deeply stained chromatin structure were also found in the nuclei of lymphocytes with MAC and were not observed in lymphocytes of patients without evidence of malignant neoplasia.

In addition, a series of ancillary changes were noted. In the polymorphonuclear leucocytes deeply stained condensations of the nuclear membrane were frequently seen. Often several conspicuous projections of varying length, thickness and shape with a thin or broad base at the outer nuclear membrane were noted. In lymphocytes, the ancillary changes included an increased number of enlarged and well-defined chromatin condensations at the nuclear membrane. Occasionally abnormally shaped nuclei with a disorderly chromatin structure were present.

In monocytes of blood films from patients with malignant tumours, MAC were less frequently found than in polymorphonuclear leucocytes and lymphocytes. When present, the nuclear structure had essentially the same alteration as in polymorphonuclear leucocytes and lymphocytes. In monocytes, nuclear elongation with increased folding and/or lobulation was observed. The article includes 27 photographs. J.H.

HAEMATOLOGY

Partial Thromboplastin Time Test with Kaolin: Diagnosis of Haemophilia and Christmas Disease without Natural Reference Plasmas. Knights, Susan F. and Ingram, G. I. C. (1967), *J. clin. Path.*, 20, 616.

Deficiencies of both Factor VIII and Factor IX prolong the partial thromboplastin time, and if plasma showing a prolonged time is retested after adding either alumina-absorbed normal plasma (containing Factor VIII) or eluate from the alumina after adsorption (containing Factor IX), it is possible to differentiate between haemophilia and Christmas disease.

Practical details are given and experiments on the validity of the test are described.

New Slide Test for Infectious Mononucleosis. Davidson, R. J. L. (1967), *J. clin. Path.*, 20, 643.

Experiments have substantiated the claim that the Denco-IM test is more specific for infectious mononucleosis than other screening procedures, and its diagnostic accuracy compares favourably with that of the conventional differential absorption test.

A Simple Method for Factor V Assay. Hoffman, Mae E. (1967), *Amer. J. med. Technol.*, 33, 281.

A simple and reproducible method of assaying Factor V in human plasma is presented, which uses sodium citrate eluate from calcium phosphate adsorption of oxalated plasma as the source of prothrombin, Factor VII and Factor X.

The Laboratory Control of Anticoagulant Therapy: The One-Stage Prothrombin Time-Quality Control in Coagulation Procedures. Barrington, H. D. and Peterson, E. W. (1967), *Amer. J. med. Technol.*, **33**, 296.

Comparing the usefulness of 0.1M sodium oxalate and 3.8% sodium citrate for the anticoagulation of blood samples used for prothrombin time estimations, it was shown that in oxalate Factor V activity diminishes rapidly, commencing at 15 minutes after the withdrawal of the sample. Sodium citrate, on the other hand, seems to stabilise Factor V and does not result in erroneously prolonged prothrombin times.

The study also showed that the time of incubation and the temperature of incubation are critical in the control of the performance of the one-stage prothrombin time.

A Simple Method for the Rapid Detection of Hypofibrinogenemia. Scarey, R. L., Simms, N. M. and Low, E. M. Y. (1967), *Amer. J. med. Technol.*, **33**, 326.

This method proposes the determination of fibrinogen levels in terms of the height of centrifugally sedimented columns of precipitate formed in plasma incubated at 56°C. to 60°C.

The technique is simple and yields fibrinogen values which compare favourably with those derived by a conventional method.

A New Test for the Detection of Infectious Mononucleosis. Dann, T. C. (1967), *Brit. J. clin. Pract.*, **21**, 511.

The use of RBCs stabilised by formaldehyde as immunological indicators has been described by Wide (1962). A series of stabilised RBCs from several species was evaluated in the diagnosis of infectious mononucleosis, and horse RBCs were found to be the most sensitive and specific. The test used in the present study (the Denco-IM Test) is an immunological one using a 4% saline suspension of formalised horse RBCs as antigen. One drop of this suspension is added to a drop of the patient's serum on a glass slide and mixed with a wooden stick. It is rotated for two minutes and read for agglutination within this time using indirect lighting from below over a dark background. A saline control may be carried out at the same time. Finely granular patterns are seen regularly in the controls and occasionally with control sera; this degree of granularity constitutes a negative reaction. Positively reacting sera produce coarse agglutination as opposed to fine granulation. Care must be taken in reading if the observer is not used to dealing with immunological tests. This is especially true in cases with a low titre of antibody.

Blood from 139 patients was tested. The new test was found to be rapid, sensitive and virtually 100% specific. Moreover, it requires no special skill, training or apparatus. It does not require inactivation of complement, nor does it require fresh sheep RBCs. The formalised horse RBCs are stable for at least a year at 4°C. J.H.

The Segmentation of Polymorphonuclear Neutrophils. The Conditions in Hypovitaminosis B_{12} and Hypersegmentation. Edwin, E. (1967), *Acta med. scand.*, **182**, 401.

The author suggests a new method for the evaluation of hypersegmentation. The aim of the present investigation was to find a sensitive method which, compared with earlier methods, would be less affected by various sources of error. It was felt that these requirements might conceivably be met if hypersegmentation could be expressed by a ratio between two cell groups. The cell groups should be closely related morphologically so that staining technique and thickness of films would influence the two groups in the same way, the ratio thereby remaining constant.

Twenty-five patients with a serum B_{12} below 150 μg./ml. were examined and what seemed typical was a disproportionately large increase in the number of cells with 5 or more lobes. This disproportionate increase in cells with 5 or more lobes is best seen by comparing this group with the group of cells having 4 lobes.

The results led the author to establish the following formula: Segmentation Index = granulocytes with 5 lobes or more X 100/granulocytes with 4 lobes. The normal value was found to have a mean of 8.5 with a standard deviation of 5.11; a range of 0-16.9 was observed in normal individuals.

The segmentation index was found to be a more sensitive method than the lobe average or counting the number of cells with 5 or more lobes. Using the segmentation index, 64% of patients with serum B_{12} less than 150 μg./ml. show hypersegmentation.

Blood films were taken in the ordinary way. To obtain a fairly even blood film a square 16 x 16 mm. from the thinnest part of each film was marked off. The counting was performed within this square, 500 granulocytes being counted. Acceptance of two parts of a nucleus as independent lobes required that the bridge between them should not exceed 50% of the largest diameter. Sex chromatin was not counted. No lower limit, however, was put on the size of the lobe.

All films were stained with May-Grunwald-Giemsa; the greatest possible light intensity and 1000X magnification were used. J.H.

HISTOPATHOLOGY

A Histochemical Study of Gaucher's Disease and Niemann-Picks Disease. Lake, B. D. (1967). *J. roy. mic. Soc.*, 86, 417-425.

In an effort to distinguish between Gaucher's disease and Niemann-Pick's disease, cryostat sections of fresh frozen biopsy and necropsy tissues were subjected to several staining and histochemical methods. These include H and E, PAS, sudan black coupled tetrazolium, Baker's acid haematin, acid and alkaline phosphatase and numerous methods to demonstrate enzymes. One of the most useful techniques was Baker's acid haematin. Gaucher cells are always PAS positive. Niemann-Pick cells can be negative or positive and this varies from case to case and from organ to organ in the same case. D.T.

A Congo Red staining Method for Epoxy-Embedded Amyloid. Tsuranobu Shirahama and Alan S. Cohen., (1966), *J. Histochem. Cytochem.*, 14, 725-29.

Tissues were fixed in buffered 1% osmium tetroxide or buffered 3% glutaraldehyde (or 4% paraformaldehyde followed by 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon or Araldite. Thick sections (0.5-2.0 microns) were floated on a drop of Congo Red solution (0.5-1% Congo Red in 0.1N sodium hydroxide) and incubated at 45° C. for 60-120 minutes followed by several changes of distilled water. The sections were then placed on a glass slide and dried at 50-60° C. If required the sections were counterstained with Azur II methylene blue for 20-30 seconds. Amyloid was stained clear red and showed strong characteristic green birefringence under the polarisation microscope.

D.T.

A New Fluorescent Method for the Histochemical Demonstration of Nucleic Acids. Burns, J. and Leveson, J. E., (1966)., *J. roy mic. Soc.*, 86, 167-175.

Numerous tissues were fixed in formalin for 10-16 hours or Carnoys for 4-6 hours. Paraffin sections (4-6 microns) were brought to water and treated with 10% aqueous formaldehyde for 20-30 minutes, then rinsed in tap water. The slides were placed in a glass staining dish containing a few drops of bromine, the dish was then sealed for 5-10 minutes after which time the sections were washed in tap water, dehydrated, cleared and mounted in D.P.X. The sections were examined under u.v. light using a UG 1 exciter filter and a K430 barrier filter. DNA and RNA exhibit an intense blue white fluorescence.

Extraction methods using DNAase, RNAase, perchloric acid, etc., and comparisons with other DNA and RNA staining techniques were used to show that the method is specific for nucleic acids. D.T.

MICROBIOLOGY

The Diagnosis and Localisation of Urinary Infection. Smart, J. G. (1967), *Ann. R. Coll. Surg.*, **41**, 283.

In regard to urinary infection it may be said that bacteriological techniques have been greatly improved, but these techniques are only as good as the standard of specimens supplied allow them to be. Bacteriologists should be encouraged in their efforts to adopt the semi-quantitative techniques, and, where necessary, the fully quantitative method. Contamination causes little difficulty in midstream specimens from the male, but in the female a high standard of technique is required and nursing staffs should be encouraged to attain and maintain it. In some hospitals it is still the practice to examine the centrifuged deposit and if the number of WBCs in the low or high power field is few the urine is regarded as uninfected and is not cultured. Estimating the number of WBCs per low power field is grossly inaccurate and enumerating them per high power field is also a coarse evaluation, owing to variation in the volume of urine excreted, variation in the amount centrifuged and in the thickness of the sediment beneath the coverslip. Furthermore, it has been shown that significant bacteriuria may occur without pyuria. There seems little doubt, however, that for general clinical purposes the estimation of the number of WBCs per cmm. of urine, using a counting chamber, is of value and this method is gradually extending in British hospital practice. Up to 3 WBCs/cmm. is considered normal; more than 10 is correlated with pyuria.

In the present work all specimens were dealt with by the laboratory within one hour of being passed. Bacteriological methods employed are described. J.H.

A Practical System for Identification of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii*, and *Mycobacterium fortuitum*. Tsukamura, M. and Tsukamura, S. (1967), *Scand., J. resp. Dis.*, **48**, 58.

It has been shown theoretically that, as the key for grouping of a given bacterial strain population into two parts, a combination of more than three tests is needed. Since there are always deviations in the bacterial character, the use of only one or two tests is uncertain for grouping. In the present study the authors utilised keys consisting of four to six characteristics.

The chief medium was Ogawa egg medium, to which was added one of the following agents: hydroxylamine hydrochloride, 8-azaguanine, sodium salicylate, sodium p-aminosalicylate, sodium thioctate, or thiophen-2-carbonic acid hydrazide. In addition, two synthetic media, nitrate medium and Sauton agar containing sodium salicylate, and five biochemical tests, niacin, nicotinamidase, three-day arylsulphatase, three-week arylsulphatase, and nitrate reduction, were used. J.H.

MICROSCOPY

Filters and Their Use in Microscopy. Freere, R. H., (1967), *Lab. Pract.*, **16**, 1110.

This article presents a survey of the main types of filters used in microscopy, with a brief discussion of their construction, applications and advantages. The use of colour and interference filters is considered in some detail, while consideration is also given to the role of these and other filters in special techniques such as black and white and colour photomicrography, polarisation, light absorption and interferens. (Author's abstract.) D.T.

The Detection and Treatment of Bacteriuria in Pregnancy — An Essential Part of Antenatal Care. Carroll, R. and MacDonald, D. (1967), *J. Ir. med. Ass.*, **60**, 115.

Approximately 6% of pregnant women excrete a significant number of bacteria (in excess of 100,000 per ml.) in their urine and it has recently

been shown that two out of every five of these develop pyelonephritis. When bacteriuria is eliminated the incidence is reduced to less than one in twenty. It has been suggested that the incidence of toxaemia is also higher and that premature delivery and perinatal death are more frequent. Patients with significant bacteriuria do not have symptoms and can only be detected by screening. Examination of the urinary deposit for leucocytes is of no value because 40% of patients with significant bacteriuria have normal WBC excretion rates.

Since April 1965, mothers who attend the National Maternity Hospital are screened and those with significant bacteriuria are treated. It takes less than one hour to process 50 cases by the filter paper technique and 20 can be screened on a single MacConkey agar plate. Cleaning the vulva is not an important preliminary and contamination can be almost eliminated by careful instruction.

It is important that there is a reasonable volume of urine in the bladder, otherwise there is difficulty in obtaining a midstream specimen because of insufficient urine to flush the urethra. The container is a waxed carton with a capacity of approximately 4oz, similar to an ice cream carton (mono container, No. 2). The surfaces are free from bacteria and sterilisation is not required. The specimen must be refrigerated but can be stored at 4°C. for 48hrs. without appreciable increase in the number of organisms.

Filter Paper Technique (Leigh and Williams, *J. clin. Path.*, 1964, 17, 498). Filter paper (Devon-Valley, No. 413) is cut into strips, 3in long and ½in wide and a fold is made across the strip ½in from one end. The strips are then sterilised. The ½in by ½in rectangle is dipped into the urine and an imprint is made on a MacConkey agar plate. The plate is incubated overnight. A growth of 30 colonies or more is regarded as evidence of significant bacteriuria.

J.H.

What's New

NEW SPECTROPHOTOMETER SYSTEMS FOR CLINICAL LABORATORIES

Unicam Intruments Limited of Cambridge, England, have announced two new uv/visible spectrophotometer systems, designed to produce single results with high accuracy on a non-automatic basis or multiple results with a high degree of automation.

The systems are based on redesigned versions of the well-proven SP600 and SP500 spectrophotometers, together with a new SP22 linear-logarithmic recorder and SP40 Automatic Sample Changer.

The SP600 Series 2 System:

In its basic form the SP600 Series 2 is a precision single beam spectrophotometer covering the range 335-1000 mμ. The spectral bandwidth is 3 mμ, and the photometric accuracy is ± 0.5% transmission. In addition to its manual mode of operation, whereby results are obtained on a potentiometric null-balance readout, the intrument is equipped with a 10 mV recorder outlet. This is designed for use with the SP22 linear-logarithmic recorder, which has switchable linear 0-10 mV and one logarithmic range of either one decade or one half-decade (0-1 Absorbance or 0-0.5 Absorbance). The accuracy of the recorder is compatible with that of the main instrument.

Multiple sampling is achieved using the SP40 Automatic sample changer. This new device accepts up to 50 samples and feeds them sequentially into the SP600, where each is measured in a cell of minimum volume 0.6 ml at 10 mm pathlength, and after measurement is returned to its original container. The cross-contamination, even without flushing, is very low, being of the order of 0.3% of the cell volume. In general this is negligible. The sample changer incorporates a programme which can control the SP22 recorder or any other readout device. Empty sample positions in the sample rack are not sampled. The sampling time per sample is of the order of 35 seconds. As there is no loss of sample, measurements can be made sequentially at several wavelengths, if desired, simply by resetting the wavelengths and repeating the run on all the samples.

The SP40 Accessory incorporates a system for adjusting the volume of the sample fed into the SP600 so that cell pathlengths greater and less than 10 mm may be used if desired.

Other accessories for kinetic measurements and microsamples are also available.

Typically the SP600 Series 2 System can perform measurements on 400 samples in the course of an eight-hour working day.

The SP500 Series 2 System:

This versatile and accurate analytical system is based on a new version of the SP500 Spectrophotometer, which has a range of 185-1000 mμ and a spectral bandwidth of between 0.5 and 2 mμ depending on the wavelength. The accuracy of the photometer system is ± 0.3% transmission, and the solid state electronics are designed for high stability. Sophistication such as automatic lamp changeover is built-in.

The instrument has three forms of readout — potentiometric null-balance, direct-reading as a built-in meter, and recorder readout. It may therefore be used with the SP22 linear logarithmic recorder and the SP40 Sample Changer as in the same manner as the SP600 Series 2 instrument.

The SP500 Series 2 Instrument is particularly adapted for kinetic measurements at constant wavelength. Further accessories designed for this work include a programmer unit, an automatic cell changer and a thermostatted cell holder. With these kinetic or flow-through measurements may be made automatically on three samples against one reference, either repetitively every few seconds or on a time delayed basis. The Programmer may be remote controlled, making it very suitable for use with fraction collectors.

SERUM LOW DENSITY LIPOPROTEIN ASSAYS

Fast and reliable results in the assays of low density lipoproteins are now possible with a new kitset manufactured by *British Drug Houses Ltd.*

Estimation of low density lipoproteins has hitherto been associated with time consuming, complicated ultra-centrifuge and electrophoresis methods. Now the new BDH Lipoprotein Set will give reliable results in only ten minutes, using a simple colorimeter and standard laboratory apparatus.

The total low density lipoprotein measured correlates with both serum cholesterol and serum triglyceride levels, and is of diagnostic value in thyrotoxicosis, steatorrhoea, myxoedema, nephrotic syndrome and xanthomatosis etc.

The kitset contains all the necessary reagents for 100 determinations.

NEW REAGENT STRIPS FOR BLOOD UREA

Azostix reagent strips by the *Ames Company* provide a rapid screening test for the semi-quantitative enzymatic determination of blood urea. The test is designed primarily for screening and is not intended to replace more precise quantitative procedures.

Details from *Potter & Birks (N.Z.) Ltd.*, P.O. Box 11-125, Ellerslie.

NEW TEST FOR INFECTIOUS MONONUCLEOSIS

A new slide test for infectious mononucleosis is announced by *Ortho Diagnostics* of Raritan, N.J. Marketed under the name *Monospot*, this test provides a one-minute differential slide test for the disease.

The test embodies differential absorption of the patient's serum, and the results are reported to be comparable with that in the conventional Paul Bunnell test. A positive control serum is included with every batch.

Details from *Johnson & Johnson (N.Z.) Ltd.*, P.O. Box 11-125, Ellerslie.

AUTOMATIC DILUTION EQUIPMENT

New from the Instruments Division of *Warner Chilcott Laboratories* is an instrument for accurately pipetting small volumes of liquid.

Called the *Auto-Spenser*, this instrument operates at great speed and gives greater reproducibility than conventional manual pipetting procedures. The adjustable pumps can be pre-set to dispense from 20 µl. to 250 µl. of sample and from 0.5 ml. to 5.0 ml. of reagent. The liquids are entirely contained in a chemically inert Teflon system.

Details from *Wm. R. Warner & Co. Ltd.*, P.O. Box 430, Auckland.

The Junior Essay Competition, 1968

The Council invites entries from unqualified members of the Institute for the two sections of the Junior Essay Competition.

A prize of FIFTEEN DOLLARS will be paid to the author of the best essay in each section, and the winning essays will be published in the JOURNAL.

Instructions for intending entrants, who must be financial members of the Institute, are obtainable on request from the Secretary.

Essays should be submitted to the Editor of this journal, and must reach him no later than June 4, 1968.

The Library

List of Periodicals Received

Librarian: D. S. Ford, Blood Bank Laboratory, Dunedin Hospital

Amer. J. med. Technol. Volume 33, No. 4 July-August 1967.
Contents: Bone Decalcification Expedited by Ultrasonic Sound; Serum Creatine Phosphokinase—A Useful Tool in Muscle Disease; Serum Creatine Phosphokinase in Myocardial Damage; A Simple Method for Factor V Assay; Bacteriological Procedures for the Small Hospital Laboratory—A Rapid Method for Isolation and Identification of Staphylococci, Streptococci and Gram-negative Bacilli from Clinical Material; The Laboratory Control of Anticoagulant Therapy—One-Stage Prothrombin Time Quality Control in Coagulation Procedures; The Effect of Bilirubin Concentration on Determination of Serum Albumin: An Evaluation of Media for Differentiating Non-fermenting Gram-negative Bacteria; Post-mortem Backflow Studies; A Simple Method for Rapid Detection of Hypofibrinogenaemia: Plasma Clotting—A Tool for Cytology; Coverslip Staining Rack; A Combination Method for Demonstrating Starch and Gelatin Hydrolysis; Revision of a Method for Performance of Total and Differential Cell Counts in Cerebrospinal and Serous Fluid; Interference of Lysogenic *Shigella flexneri* with Antimicrobial Disc Susceptibility Readings; Present Status of the Good Antigen and Anti-Good Antibody; Management and Medical Technology.

Volume 33, No. 5, September-October 1967.
Contents: The Determination of Serum Magnesium: Automated Fluorometry *vs.* Atomic Absorption; An Evaluation of a Gel Filtration-Spectrophotometric Method for Spinal Fluid Protein; Rapid Determination of Urea Nitrogen in Serum or Plasma Without Deproteinisation; Serial Lactic Dehydrogenase and Glutamic Oxaloacetic Transaminase Automated Analysis; A Rapid Test for Lysine Decarboxylase Production by Enterobacteriaceae Cultures; Effect of Incubation Temperature on Enzyme Production for Lysine Decarboxylase and Cytochrome Oxidase Tests; Pasteurella Meningitis—A Review of the Literature; Quality Control in Bacteriology Through Media Monitoring; Polyester wax as an Embedding Medium for Serial Sectioning of Decalcified Specimens; Cryptococcosis Due to an Unusually Slow-Growing Organism; Concurrent Determination of Urinary 17-Ketogenic and 17-Keto Steroids; Refresher Programs for Medical Technologists; Developing an Inservice Education Program for Clinical Laboratories; A Simplified Method for the Determination of Skin Glucose; Long Term Storage of *Diplococcus pneumoniae.*

Ann. Med. exp. Biol. Fenn. Volume 45, No. 1. 1967.
Volume 45, No. 2. 1967.

Arch. Inst. Pasteur hellen. Volume 12, No. 1/2 1966.
Les Shigella en Grece en 1965-66; Determination des Types Biochemiques des *Shigella sonnei* isolees en Grece en 1966; Recherche des Salmonella dans les Ganglions Mesenteriques des Porcs et des Bovins aux Abattoirs d'Athenes; Note sur la recherche de Brucella dans les Ganglions Mesenteriques des Animaux de Boucherie; Recherche des Salmonella dans les Selles de Certains Animaux; Differentiation Rapide des Enterobacteries par Utilisation des Milieux Lactoses Concentres; Etude immunologique de l'Antigene A_x chez une Famille Grecque.

Aust. J. biol. Sci. Volume 20, Nos. 5 and 6 October and December 1967.

Canad. J. med. Technol. Volume 29, No. 4. August 1967.
Contents: Antigen, Antibody and Complement; The Use of *Pathotec* Strips in Medical Bacteriology; Application de la Chromatographie en Couche Mince; Haemolytic Disease of the Newborn due to Antibodies other than ABO and Rh; A Simplified Mallory's Phosphotungstic Acid-Hematoxylin Stain; A Simplified Toluidine Blue Stain for Mast Cells.

Volume 29, No. 5. October 1967.
Contents: Le Diagnostic Bacteriologique des Infections Urinaires; Screening for Glucose-6-Phosphate Dehydrogenase Deficiency; A 17-Ketogenic Steroid Procedure.

Filter. Volume 39, No. 3. September 1967.
Contents: Rapid Methods for the Identification of Selected Substances in Biological Specimens; A Practical Micro Blood Sugar; Some Information to Help Obtain Reliable Results in Performance of the BSP Test; Prevention of Rh Isoimmunisation in Rh Negative Mothers; Observations Regarding the Usefulness of a Rapid Heterophile Procedure; The Future Laboratory Technologist?

J. Amer. med. Technol. Volume 29, No. 4. July-August 1967.
Contents: Donor Motivation in a Volunteer Blood Program; Technical Characteristics of Commercial Instruments for Determining the Hematocrit; Technical Characteristics of Commercial Atomic Absorption Spectrophotometers; Clinical Chemistry Nomenclature and Unitage; Ratification of Original Rh-Hr Nomenclature—The End of a Long-Lasting Dispute.
Volume 29, No. 5. September-October 1967.
Contents: T-3 or PBI; An Epidemiological Survey of Rabies in Morocco; The Value of Non-Protein Nitrogen (NPN) in Clinical Medicine; Inborn Errors of Metabolism A Brief Review.

J. med. Lab. Technol. Volume 24, No. 3. July 1967.
Contents: Rapid Identification of Coliform Organisms from Extra-intestinal Infections; A New Selective Medium for *Streptococcus pyogenes* and other Streptococci; Results of a Questionnaire on the Estimation of Magnesium in Body Fluids; A Weak Sub-group of B, B^m, in a Canadian Family; An Apparatus for Measurement of Platelet Aggregation, Prothrombin and Other Clotting Investments; An Evaluation of Pagano-Levin Medium in a Quantitative Study of *Candida albicans*—Preliminary Communication; A Comparison of the Triphenyl Tetrazolium Chloride (TTC) Test and a Modified Nitrate Reduction Test for Bacteriuria; Modified Technique of Polyethylene Glycol-Wax Embedding; Haemolysin Production by *Corynebacterium pyogenes;* Stability of Antituberculosis Drugs in Lowenstein-Jensen Medium; Distorting Effects of Histological Processing—An Historical Review; Modifications to Centrifuge Controls to Allow Automatic Platelet Separation from Diluted Blood; A Container for Cold Urine Collection; A Simple Tissue Culture Chamber Suitable for Extended Phase Contrast Microscopy of Tissue Cells; An Embedding Mould for Small Pieces of Tissue; An Aid to Karyotype Preparation in Chromosome Analysis.
Volume 24, No. 4. October 1967.
Contents: New Words in Biology; Human Studies in the International Biological Programme; Detection of Dexamphetamine Sulphate in Urine; Determination of Total Urinary Nitrogen on the AutoAnalyser following Kjeldahl Digestion; Automated Simultaneous Blood Glucose and Urea Determination; A Colorimetric Assay for Serum Phenylalanine; A Method of Preparation of Cartilage for the Study of its Enzymes; Methyl Green as a Stain for Amyloid; Storage of Bacteria in Liquid Nitrogen; Comparison of Platelet Count Methods; Polycell as an Embedding Medium for Large Sections; A Rapid Thin Layer Chromatographic Screening Method for the Increased Urinary Excretion of 4-Hydroxy-3-Methoxymandelic Acid (V.M.A.); A Semi-micro Serological Centrifuge; A Method of Separation and Storage of Plasma for AutoAnalyser Analysis; A Labour-saving Method of Collecting Cell-free Serum in Plastic Containers.

Lab World. Volume 18, Nos. 8-12. August-December 1967.

Med. Technol. Aust. Volume 9, No. 3. July 1967.
Contents: Classification of Leukaemias and Special Stains used in their Identification; Some Aspects of Laboratory Design; Contrasting Stains in Electron Microscopy; Infections of the Urinary Tract, Their Importance and Laboratory Methods of Diagnosis.

Volume 9, No. 4. October 1967.
Contents: The Small High Speed Digital Computer—A Laboratory Tool; A Method for Embedding Vesical Calculi in Polyester Resin; Some Aspects of Environmental Changes on the Fine Structure of the Blood Platelet; Streptococcal Groupings—A Simplified Approach; A Quick and Accurate Method of Checking Pipettes.

Microbiologia (Buc.) Volume 12, No. 3. May-June 1967.
Selected contents: A Factor of Resistance to Antibiotics (The R Factor); Incidence of Anti-Coxsackie Antibodies in Laboratory Animals*.

Volume 12, No. 4. July-August 1967.
Contents: The Factor of Resistance to Antibiotics; Problems Derived from the Study of Altered Forms of Bacteria; Incomplete Antibodies; Contributions to the Study of Incomplete Antibodies in Typhoid Fever after Treatment with Chloramphenicol*; Studies Concerning Actual Clinical, Aetiological and Epidemiological Aspects of Intestinal Infections*; Elucidation of the Mechanism of Contamination with *Pseudomonas aeruginosa* of Dental Devices by the Combined Use of Lyso-and Serological Typing*: Importance of the Anti-microbial Factor in Anti-diphtheric Immunity*; Efficiency of Anti-helminthic Actions in Stone-quarriers in the Area of the Cluj Railway District*; Considerations on Clinical Manifestations of Strongyloidosis; *Corynebacterium ulcerans;* Use of Tinsdale Medium in the Bacteriological Diagnosis of Diphtheria*: Changes in the Method of Bacteriological Diagnosis of Diphtheria*.
[Articles in Rumanian. * = English summary.]

New Instanbul Contr. clin. Sci. Volume 9, No. 1. 1966.
Selected contents: A Study on the Effect of Oestriol Succinate on Blood Coagulation; Thrombocytosis with Impaired Platelet adhesiveness and Platelet Factor 3 Availability; Agnogenic Myeloid Metaplasia with Megaloblastic Erythropoiesis.

N.Z. Hospital. Volume 20, Nos. 1, 2. September, November 1967.

N.Z. med. Ass. News. Volume 3, No. 16. Christmas 1967.

Ortho Diagnostic Reporter. Volume 2, No. 1. 1967.
Contents: Erythropoiesis; DC^{We}/dce ($R_1^{W}r$) Patient Producing Anti-C; Minot—Beneficiary of a Life-Extending Discovery who Made his Own Life-Extending Discovery; Spherocytosis.

Volume 2, No. 2. 1967.
Contents: Blood Volume Determination; Importance of Antibody Identification; Paul Erlich—Doktor Phantasus' Magic Bullet; Antibody Titration.

Volume 2, No. 3. 1967.
Contents: Abnormal Coagulation States; Hemolytic Disease of the Newborn due to Anti-B; Fernand Widal—Original Researcher—Brilliant Diagnostician—Remarkable Teacher; Elution Techniques.

Volume 2, No. 4. 1967.
Contents: Catabolism of Immunoglobulins; Polyagglutinability—A Rare Cause of Incompatibility in the Minor Crossmatch; Robert Koch—Different Bacteria Cause Different Diseases; Leukoagglutinins.

Rev. viernes Med. Volume 18, No. 2. May-August 1967.

S. Afr. J. med. Lab. Technol. Volume 13, No. 2. June 1967.
Contents: Exfoliative Cytology—A Laboratory Viewpoint.

Volume 13, No. 3. September 1967
Contents: The A Subgroups of the ABO Blood Group System.

Tonic. Volume 5, Nos. 2 and 3. 1967.

Suggestions for Speakers

It is a source of great concern to the Council that each year at the Annual Conference some speakers occupy a greater time than is allotted to them, resulting in curtailment of discussion and sometimes in other speakers being unable to deliver their papers.

Accordingly, it has been decided to reprint the suggestions for speakers which appeared in the July 1966 issue of the *Journal.*

PREPARING A PAPER

A scientific paper intended for spoken exposition should never be prepared in the same form as one intended for publication in a journal. To attempt to use the same phraseology when conveying ideas by means of the spoken word as when presenting them in print will often bore the audience. Moreover, data which can be satisfactorily presented as tables in print will convey nothing but a meaningless jumble of figures unless it is translated into the form of graphs for projection on the screen.

Long dissertations are not generally acceptable to a captive audience, and it may be that if the subject matter of the paper is not capable of being condensed into a talk lasting fifteen minutes at the most, it would perhaps be more suitably presented in print. In any case, it is necessary only to give an outline of the subject when delivering a paper to an audience. Future publication, with greater detail and a presentation of all relevant data will enable closer study at leisure.

The introduction should be *brief*, giving the essentials of the background and purpose of the investigation, with as little time as possible being devoted to a survey of earlier work. The citation of numerous references should be avoided. The theme should then be developed presenting the actual experimental work carried out, but omitting detailed descriptions of methods, and then proceeding to a presentation of the results. Results not contributing to the final conclusions should be omitted. A discussion of relevant results should then follow, with a concise summary of the conclusions drawn from them and their theoretical implications.

REHEARSING THE DELIVERY

It is extremely discourteous to other speakers to over-run the time allotted. Accordingly, it is essential for the speaker to rehearse the delivery of his paper with a watch, allowing a certain margin for incidental comments and disturbances, and time for the presentation and explanation of slides. If necessary, the paper should be trimmed down to fit the time allotted, and every speaker should know, when he stands up, exactly how long it will take him to complete the delivery of his paper. Time for questions would normally be allowed for in the planning of the programme and the duration of questioning would be at the discretion of the chairman.

It is always best if the paper is *not* read directly from a manuscript, although it is certainly permissible to have notes. In the interests of spontaneity the speaker should attempt to memorise the substance of his paper, especially the discussion. A manuscript generally needs to be prepared in order to time the delivery of the paper beforehand, and by underlining headings and important passages in red ink, the speaker can equip himself with reminders so that he can consult the manuscript if he loses the thread and is unable to proceed without so doing.

The size of the hall and its acoustic qualities will have a bearing on the tone of voice the speaker will adopt, but he must take pains to ensure that he is audible to everyone present. The voice should be directed towards the rear of the room and not at the first few rows of chairs. If using a blackboard, the speaker should refrain from speaking when his back is to the audience and should endeavour to avoid concealing the board with his body while writing. When explaining slides, only matter being illustrated by them should be treated.

The substance of the paper should be easy to understand, without being unscientific, and it may help to repeat the gist of the main argument in different words. To keep the attention of the audience it is essential to avoid speaking in a monotone. Nervous gestures, fidgeting with papers and pacing should be avoided.

ILLUSTRATIONS

A paper can be illustrated either by diagrams drawn on white paper or on a blackboard, or by lantern slides. Whichever method is used, the amount of lettering should be kept to the barest minimum necessary. Tables should be brief, showing results clearly and requiring no elaborate explanation. When using diagrams previously drawn, these should be on a card or pinned to boards and not in the form of unmanageable rolls of paper. The lettering should be bold enough to be clearly legible at a distance.

The speaker should co-ordinate the visual illustrations with the spoken matter. In this connection, slides are not always the best method of illustrating isolated points, since their projection requires that the lights be switched off and on several times during the delivery of the paper. If using slides at all, pains should be taken to ensure that the curtains are drawn at the start, that the projector is in focus and that the picture fits the screen. The slides should be placed in order and in such a way that they can be projected the right way up the first time. The manuscript should be marked to denote points at which slides are to be projected, and a system of signals should be arranged with the projectionist to ensure that the speaker's instructions do not interrupt the flow of his delivery. Slides should never be used merely for the sake of using them.

ANSWERING QUESTIONS

It is always encouraging when members of an audience signify interest by asking pertinent questions afterwards. These should be dealt with as briefly as possible, using the blackboard if necessary, but avoiding long dissertations on matters not dealt with in the paper. If the question is incapable of being answered in fairly simple terms, it may be advisable for the speaker to invite the questioner, and anyone else interested, to meet him privately afterwards.

PUBLICATION IN THE *JOURNAL*

If a paper is worth delivering to a conference, it is also worth placing on permanent record in the Institute's official journal. It may be necessary to present the subject matter in a slightly different way for this purpose, but it would help to maintain the *Journal's* standard if authors would always be prepared to submit their work for publication following the Conference.

Obituary

JOHN LESLIE ALLEN

John Leslie Allen died in an accident at the Chateau while touring at the age of 30.

John obtained his Certificate of Proficiency in medical laboratory technology and graduated B.Sc. simultaneously. He performed biochemical work in various laboratories of the Auckland Hospital, and while working in the Medical Unit laboratory developed an improved biochemical assay of chloromycetin, which has been applied in several important cases.

Lately, he was attached to the staff of the Serology Department at the Central Laboratory, and was engaged on routine and research.

John Allen was a most conscientious and very devoted worker, who will be sadly missed.

A.F.

Wellington Hospital Board Examinations in Histology and Cytology

Held between November 29 and December 1, 1967.

HISTOLOGY—Written Paper.

Time allowed: 3 hours.

Five questions to be attempted. All questions carry equal marks.

1. State the principles of two methods of decalcification. Detail the steps you would take to prepare a piece of bone for sectioning.
2. Draw a diagram to illustrate the path of light through:
   a. The binocular head of a microscope; b. An Abbe condenser; c. A dark ground condenser; d. Nicol prisms.
3. What is haematoxylin?
   Give the formulae of four haematoxylin solutions and state the advantages and disadvantages of each.
4. Define and give one example of each:
   a. Mordant; b. Accentuator; c. Progressive staining; d. Vital staining; e. Metachromasia.
5. What stain or staining technique is associated with the following names:
   Mallory, Heidenhain, Lendrum, Gram, Bielschowsky. Write a brief biography of one of these men.
6. How would you prepare, from the fixed specimen, a paper mounted section of a whole lung.

HISTOLOGY—Practical

Fifteen minutes will be allowed each candidate, in turn, to cut the sections required for question 1. Fifteen minutes will also be allowed, in turn, to cut the sections required for question 2.

Each section stained (Questions 1-5) is to be labelled with the candidate's name and with the block or section letter.

In questions 1-5 give the formula of the stain or stains used and the staining times.

1. a. Cut at 4 μ, a section from block "A"; stain with haematoxylin and eosin. *(Lymph node).*
   b. Cut a section from block "B"; stain by Glees and Marsland's modification of Bielschowsky's method. *(Cerebellum).*
2. Prepare, on the freezing microtome, a section from the tissue "C"; stain for fat. *(Lung with fat—Sudan IV).*
3. Stain section "D" by Lendrum's phloxine-tartrazine method. *(Pituitary).*
4. Stain section "E" for fungi by
   (a) Periodic Acid-Schiff method, *(Appendix with*
   (b) Gram's method. *actinomyces).*
5. Stain section "V" by Mallory's trichrome method. *(Uterus).*
6. Examine sections "W," "X," "Y," and "Z." Identify the tissue, the material demonstrated and the staining technique used.
   W—Muscle (PTAH), X—Muscle (van Gieson), Y—Liver cirrhosis (Alcoholic hyaline—Augustus Roche), Z—Spinal Cord (Loyez).

HISTOLOGY—Orals

The following subjects were traversed:

Advantages and disadvantages of a Sledge Microtome; Sharpening of knives by hand; Cedar wood oil as a clearing agent; Types of wax used for embedding; Electrophoretic decalcification of calcified tissue; Nitric acid decalcification; Formic acid decalcification; Prisms in the head of a binocular microscope; Names of different types of microtomes; Technique of embedding very small pieces of tissue; Clearing agents; Purpose of chloral hydrate in Mayer's haemalum.

CYTOLOGY—Written Paper

1. Write brief notes on the following names:—
   Koss, Schiller, Traut, Millipore.
2. Enumerate the reagents used in the Papanicolaou stain. What is the function of each?
3. Detail the preparation of Sputum smears from a *FRESH* specimen in a waxed pot to and including fixation.
4. Define:—Dyskaryotic cell; Carcinoma in-situ; Squamous metaplasia; Navicular cell.
5. What do you know of normal and abnormal vaginal flora?

CYTOLOGY—Practical

Time allowed: 4 minutes per slide.

The candidates were given 20 slides (sputa, vaginal, cervical, prostatic, gastric and pleural fluids) for diagnoses, grading and comment.

CYTOLOGY—Orals

The following subjects were traversed:—

Methods of obtaining adequate sputum specimens; Changes seen in smears with *Trichomonas vaginalis;* Oestrogen effect in the menstrual cycle; Exfoliative cytology; Different preparations of Papanicolaou stains, E.A., and O. G.; Tumour cells in peripheral blood; Squamous metaplasia; Implications of *Candida albicans* in cervical and vaginal smears.

Successful Candidates

Coe, Mrs B.; Denning-Kemp, Miss R.; Green, Miss M.; Peebles, Miss J.

Investment Comment

News from the Public Service Investment Society

The Society's tenth shop was opened recently in Invercargill.

The first shop opened by the Society was in Wellington in 1958, quickly followed by one in Hamilton (1959), Christchurch (1960), Palmerston North (1961), and Dunedin (1963). In 1965, the lease of the retail firm of J. B. Gimbel Ltd. in the Society's Auckland building having expired, the Society took over from this firm and commenced selling to members on its own account. Less than two years later, in 1966, shops were opened in Rotorua and Wanganui; and in 1967 Napier and Invercargill members were provided with their own shops.

By any standards this is a rate of progress which members can be proud of. Last year purchasing power was increased to members using the shops by approximately $300,000. This was the portion of shops' profit returned to members.

Members spend an average of $40 in the Society's shops each year. This is a figure which could be improved upon to the advantage of all concerned. If your shop can supply an article, or get it for you, it pays both you and the Society to buy it there.

Council Notes

A Council meeting was held in Wellington on Saturday, November 11, 1967. Present were Mr M. McL. Donnell (in the Chair) and Messrs J. Case, E. K. Fletcher, H. E. Hutchings, R. T. Kennedy, G. F. Lowry, B. W. Main, J. D. R. Morgan, D. J. Philip, L. R. Reynolds and C. S. Shepherd.

Applications and Resignations.

New Members

Ordinary Members

Berry, Miss M. E.	Palmerston N	Leach, R.	Tauranga
Clark, J. N. T.	Gisborne	McDonald, Miss D. L.	Rotorua
Clark, R. A.	Whangarei	McNally, N. C.	Palmerston N
Corbett, Miss L. E.	Auckland	Parkinson, A. J.	Gisborne
Ellis, Miss C.	Gisborne	Powell, J. C.	Auckland
Hunt, Miss S. A.	Tauranga	Signal, Miss T. M.	Rotorua
Keenan, E. S. M.	Palmerston N	Snowball, R. D.	Rotorua

Reinstated

Certificated Members

Van den Bemd, E. M. J.	Palmerston N	Harrison, Miss M. E.	Christchurch
		Simms, R. I. C.	Palmerston N

Elected Associate

Calvert, Mrs Y. M.	Christchurch	Schollum, Miss K.	Auckland
Elliot, J. E.	Wellington	Taylor, Miss M. A.	Christchurch
Mold, Miss M. E.	Gisborne	Willis, Miss E.	Hastings
Norman, E. S. P.	Christchurch		

Resignations

Aldworth, Miss J. M.	Auckland	Pridham, Miss A.	Tauranga
Collins, A. A.	Upper Hutt	Scoggins, Miss J.	Whangarei

Registration Fee for Conferences

The Council agreed that it would not be unreasonable for delegates to the Annual Conference each year to be charged a registration fee.

Conference Programme

In view of past difficulties created by time being overrun by speakers at annual conferences, it was agreed that the programme and appointments of forum chairmen should in future be vetted by the Council. It was also suggested that possibly the first night of the Conference could in future be available for a general discussion on all topics, providing a prelude to the A.G.M.

Examinations for Technical Assistants

Mr Kennedy reported on the sub-committee's recommendations regarding the examinations for laboratory assistants and it was decided to submit the proposals to the Director-General of Health and to the Medical Laboratory Technologists Board for comment.

Salary Negotiations

With the failure of the Government to enact legislation setting up the proposed Hospital Service Tribunal during 1967, protests had been made by the Committee of Combined Hospital Employee Organisations. A delegation had been received by the Deputy Prime Minister and it had been agreed that the N.Z.I.M.L.T. should be entitled to make representations regarding the salaries and conditions of employment of its members without having to go through the tedious and time-consuming process of the Salary Advisory Committee system.

The Deputy Prime Minister had agreed to the setting up of an interim conciliatory committee for the purpose of hearing submissions, and this meeting of the Council was called primarily for the purpose of drawing up the submissions.

Mr Hutchings made his recommendations for a complete review of the system of gradings, salary structure and conditions of employment, and after prolonged discussion and some amendment these were adopted to be forwarded to the Minister.

Negotiations are proceeding and it is anticipated that their outcome will not be long delayed.

Branch Report

DUNEDIN BRANCH

SECRETARY: D. S. Ford, Blood Bank Laboratory, Public Hospital, Dunedin.

The November 1967 meeting consisted of a tour of the University of Otago Computer Centre, where members were shown how computers can be used to obtain statistical data and to store medical records etc.

The provisional programme for 1968 is:

March: Discussion on Education and training.
April: *The Physician and the Laboratory Services.*
May: *The Surgeon and the Laboratory Services.*
June: (i) Film evening.
(ii) Annual Branch party.
July: Conference reports.
August: Meeting at Invercargill.
Sept: Annual General Meeting.

The Dunedin Branch is also organising the programme for the 1968 South Island Seminar which will be held at Timaru on 6 April. The programme will consist of short introductory talks followed by open discussion. Members who are willing to present short papers are requested to inform the Dunedin Branch Secretary by 20 March. It is hoped that the Seminar will receive the usual excellent support from all laboratories in the South Island. D.S.F.

Regional Seminar Report

Auckland Branch Seminar

On Saturday, 7 October, 1967, 120 medical laboratory technologists, local and visiting, assembled in the Gonzaga Hall, Mater Hospital, Auckland, to participate in the local branch's 8th one-day seminar, the programme for which appears below:

Morning:

Welcoming address and opening of Seminar—Mr M. Donnell.
A Broad Outline of the Function of the Gastro-Intestinal Tract —Dr A. G. Tymms.
The Microbiological Assay of Vitamin B_{12} and Folate —Mr G. McGough.
Malabsorption Syndromes—Dr R. Farrelly.
Bacteria and Gastro-Intestinal Function—Dr G. Nicholson.
The Dietary Control of Gastro-Intestinal Disorders—Miss M. Till.

Afternoon:

Eosinophilia in a Child, Caused by Toxocara canis—Mr J. Marr.
The Laboratory Diagnosis of Paroxysmal Nocturnal Haemoglobinaemia —Miss J. Grey.
Serum Chloromycetin Estimation—Mr J. Allen.
Discussion Period on the Spectroscopy of Haemoglobins —Mr D. McArthur.

Evening:

Approximately 75 persons attended a magnificent buffet dinner, completely catered for, in the Cornwall Park Tea Kiosk from 5.30 p.m. to 10 p.m.

The Auckland branch acknowledges the financial assistance of the following trade organisations in mounting this most successful seminar: Muir and Neil Pty. Ltd.; Burroughs Wellcome & Co.; S. A. Smith—Biolab. Ltd.; Scientific and Laboratory Equipment (N.Z.) Ltd.; Dominion Dental Supplies Ltd.; Ames Company; Laboratory Services Ltd.; National Dairy Association of N.Z. Ltd.; William R. Warner and Co.; Early

Brothers Ltd.; Ethicals Ltd.; E. C. Lackland and Co.; Tasman Vaccine Laboratory Ltd.; Geo. W. Wilton and Co. Ltd.; N. M. Peryer Ltd.; Townson and Mercer Ltd.; Watson Victor Ltd.

The Branch also thanks the Auckland Hospital Board for providing lunch at a very reasonable cost, and the Matron of the Mater Hospital and her laboratory staff for the venue and facilities provided, including provision of morning and afternoon teas.

Letters to the Editor

BLOOD BANK ALARM

Sir,

Having recently had the approved 48-Unit Blood Bank installed we find that the visual alarm system alone is not sufficient to attract immediate attention, and have fitted, in addition, an alarm bell which sounds if the temperature varies from the normal, which once triggered cannot be silenced until reset manually.

We would be pleased to give any further information required to anyone interested.

L. R. TAYLOR & K. H. BODDY,
Oamaru Hospital.

COMPARISON OF QUALIFICATIONS

Sir,

The letter from Mr M. R. Ford published in Vol. 21 No. 2 of the *New Zealand Journal of Medical Laboratory Technology* has just been brought to my notice. I apologise for failing to note it when the *Journal* was received by us in September.

For as long as I can remember, the qualifications of this Institute have been regarded with respect by colleagues and others in countries overseas and perhaps Mr Ford is right to think of them as symbols of status. His reference to "the ol' grey mare," which obviously applied to this Institute, was perhaps more pertinent than he thought. That "she ain't quite what she used to be" is always true of any progressive organisation; indeed, as he should have known, the qualifying system in the last year or two has undergone radical changes, providing an open-ended structure for progress to a professional qualification of degree status and beyond. It would seem that Mr MacGibbon, whose letter was published in the same issue of the *Journal,* is in favour of such a system, linked with appropriate promotion.

Some of the information given in Mr Ford's letter might have been more informative. It is true that, for the reason he stated, the recruitment of school leavers with 'A' levels in some areas is not as easy as we would wish, although there is no problem in districts where the employment prospects are less favourable. Happily, under the new qualifying system all students in training will reach 'A' level standard in appropriate science subjects, remission of part of the training period being given for possession of 'A' level passes in those subjects. His statement that "A trainee over here may sit his final examination after one year" was most misleading, that concession being available only to those awarded science degrees by approved universities of the United Kingdom. Others are required to have periods of training varying from two years to five years according to their qualifications and education attainments, or up to four years under the new system. A telephone call to this office would have enabled Mr Ford to get his facts right, to make more valid comparisons and to base his observations on reliable evidence.

Yours faithfully,
R. J. LAVINGTON.
General Secretary—Institute of Medical Laboratory Technology,
12 Queen Anne Street, London, W.1.

CONFERENCE ARRANGEMENTS

Sir,

The comments in the November issue of the *Journal* have been carefully studied to ensure that pitfalls of the past are avoided if possible. Since time is so valuable, each Chairman must allocate this commodity—especially in the A.G.M. and discussion periods — with miserly precision.

Once again, the A.G.M. will take up part of the Conference programme, but with Council's permission this would be held on Friday morning to give delegates an opportunity to discuss the remits informally on the previous day and to point out to the more voluble delegates that the business of the A.G.M. must be put through in, say, 3½ hours. (The final allocation of time will depend on the nature and number of remits submitted.)

We believe that short papers of a practical nature presented without lengthy charts and statistics will stimulate more interest and discussion, so we intend to limit the presentation of papers to ten minutes, to allow time for any salient points to be fully elaborated on by the speaker during the discussion.

When you come to Napier, you can let off steam, but not too much hot air.

T E. BROWN.
F. SMITH.

THROMBOPLASTIN STANDARDISATION

Sir,

Thromboplastin Standardisation was recently discussed at a Haematology forum held by the Christchurch Branch of N.Z.I.M.L.T. The conclusions reached were similar to those mentioned by W. J. Wilson in the last issue of the *Journal*.

The methods at present in use in the laboratories represented at the meeting are as follows:—

CHRISTCHURCH & PRINCESS MARGARET HOSPITALS: Quick's One Stage Method, using home-made thromboplastin.

PEARSON LABORATORY & GODFREY LABORATORY: Quick's One Stage, using *Simplastin*.

GREYMOUTH HOSPITAL & ASHBURTON HOSPITAL: In both these hospitals *Thrombotest* is used for controlling patients on anticoagulant therapy and Quick's test *(Simplastin)* for routine requests on non-anticoagulated patients.

Each laboratory was able to provide valid reasons for the method of choice and, quite obviously, each laboratory has selected the methods which most suit its particular requirements.

It seems therefore that to establish one particular method as a standard would be both impossible and impracticable. It was therefore decided that the Christchurch Branch of the Institute recommend the establishment of a common standard. Each particular reagent or technique would have to be correlated to a reference thromboplastin preparation.

One laboratory in the country could be established as the central reference laboratory and each laboratory participating could refer its own thromboplastin to it for correlation.

It would be interesting to know if other branches have conducted similar surveys and of their conclusions.

Our country being somewhat smaller than most should be able to achieve standardisation without too much difficulty and, as W. J. Wilson mentions, adoption of standardisation in this country could ultimately develop into an internationally controlled programme.

MARILYN M. EALES.
Princess Margaret Hospital,
Christchurch.

TRAINING TECHNOLOGISTS

Sir,

It was encouraging to find evidence of real interest and concern about the management of our Institute affairs in the last *Journal*.

The lack of opportunity to discuss education at the last Conference plainly caused concern. I regard it just as important to discuss this as technique. The quality of our technique depends on education. It is evident that the machinery for debate needs to be overhauled so that there is ample time for Council reports and discussion. There is scope for saving time; the point about "*Readers Digest* material" was well made. Most certainly let us use the evenings of Conference.

In regard to the training and qualification of laboratory assistants. this may well be desirable, but I am not convinced that this is timely until our own educational problems are settled. I am quite certain that the ability to provide training for medical laboratory technologists, which we have endeavoured to do for many years, is far more onerous and important than conducting examinations.

Ideally, the whole question of training laboratory personnel should be dealt with, keeping in mind the sound principle of an "open-ended" structure. This fashionable phrase indicates the aim of arranging examination levels so that there is the possibility of progressing from one grade to the next. In our case one could envisage laboratory assistants passing through the T.C.A. course to Intermediate level and being accepted for technologist training. The technologist's education would, and probably should, have a greater academic content to make the next transition to science graduate feasible. There is in any case a gap between school science and the theoretical background to medical technology which we all have to bridge.

In conclusion, it is worth while to consider the progressive approach of a country like Venezuela described by Mr Rush-Munro in the *Journal*, March, 1967. This is neither a large country populationwise nor a wealthy one, but there are three schools attached to universities providing a full time 4-year course in medical laboratory technology or "bioanalysis" (as they call it).

As I indicated in my last letter, the situation calls for a small slice of the cake, not the crumbs.

BOB ALLAN,
Dunedin Hospital.

Retirement of Mr D. Whillans

The Auckland branch of the New Zealand Institute of Medical Laboratory Technology (Inc.) wishes to notify all Institute members that the annual one-day seminar for 1968 has been advanced to Saturday, 25 May, 1968, to coincide with the retirement of Mr Douglas Whillans from active duties with the Auckland Hospital Board's Laboratory Services.

It is intended that the buffet dinner which follows the seminar will incorporate an official farewell ceremony and presentation from past and present staff members of the Auckland Hospital Board's Laboratories and other interested parties.

Any technologist who may be interested in attending this function and/or contributing toward the presentation should communicate with the Secretary of the Auckland branch forthwith, indicating the course of action contemplated. Accommodation can be arranged for visitors provided they state the type of accommodation required and an approximate tariff limit.

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References: 1. Wilkinson, J. F.; Nour-Eldin, F.; Israels, M. C. G., and Barrett, K. E.: **Lancet** 2:947 (Oct. 28) 1961.
2. Hicks, N. D., and Pitney, W. R.: **Brit. J. Haem.** 3:277, 1957.
3. Langdell, R. D.; Wagner, R. H., and Brinkhouse, K. M.: **J. Lab. & Clin. Med.** 41:637, 1953.

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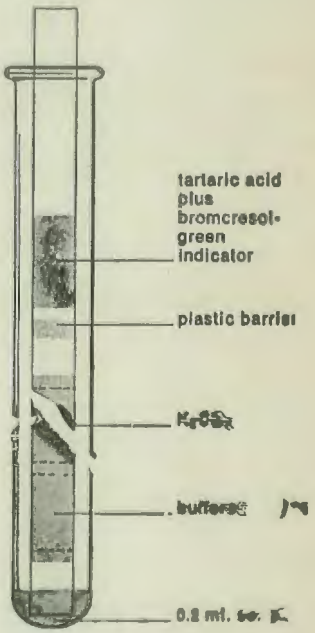

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