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JOURNAL

OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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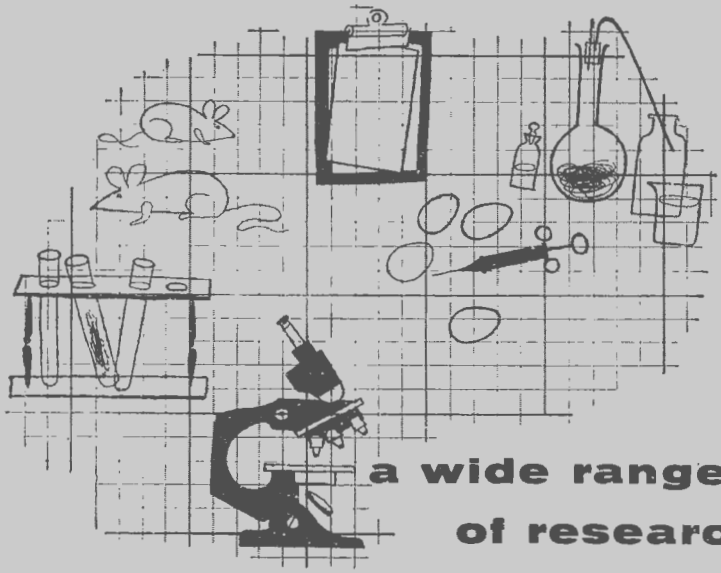

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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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PAPER CHROMATOGRAPHY IN THE HOSPITAL LABORATORY

MISS J. MATTINGLEY
(Pathology Department, Wellington Hospital)

Prior to 1941, chemical investigation of proteins and polysaccharides was very difficult because the methods available for separating and identifying their fundamental units, the amino-acids and sugars, were quite inadequate. They were tedious and required large amounts of material which were not always available. In 1941 two English workers, A. J. P. Martin and R. L. M. Synge, developed a process called Partition Chromotography. They poured an amino-acid mixture on to a column of silica-gel wet with water, then added chloroform. The individual amino-acids, having differing solubilities in water and chloroform, were carried at different rates through the column, each being collected as it emerged from the bottom of the column. In 1944 Consden, Gordon and Martin substituted a strip of filter-paper for the silica-gel column. Thus Paper Partition Chromatography was born and it has been developed and adapted for many fields of chemical analysis.

Essentially the method is as follows; near one end of a strip of paper is put a spot of e.g. mixed sugar solution. The paper is suspended in a tank containing a solvent. The solvent when on the bottom of the tank runs up the paper, giving ascending chromatography; when it is in a trough at the top of the tank and travels down the paper, we have descending chromatography. Either way, the paper is so placed that the spotted end touches the solvent which travels along the paper by capillarity, carrying the spot with it. Due to differing solubilities and other physico-chemical features, the individual components of the mixture are carried at different rates and thus separate out as discrete spots along the paper. This takes some hours. The paper is then taken out and dried, then stained to show up the spots.

The apparatus required is simple. Various things have been used for tanks; boxes of wood or slate or glass, oil-drums, drain-pipes, measuring cylinders, battery cases, museum jars. The essential requirements are a non-reactive surface and a tight-fitting lid. The solvents commonly used attack metals and plastics and are absorbed by wood. Waxing of surfaces is usually unsatisfactory. Glass is best and has the advantage of allowing the run to be observed. If the top edges of the tank are ground level, a sheet of glass makes a good lid.

To support the paper in the tank for ascending methods, glass rod bent strategically will support horizontal glass rods on to which the papers can be clipped with glass or metal clips. Or a length of cotton knotted through each upper corner of the paper

and taken out over the top of the tank can be jammed in place with the grease between tank and lid. For the descending method replace the horizontal rods with a trough to contain the solvent. The upper end of the paper is kept in place by a weight such as a glass rod or microscope slide. The other end of the paper hangs free and the solvent drips off the end.

The solvents are usually organic solvents partially miscible with water. They must be pure. Much early work was done with collidine and lutidine but these are difficult to get pure and are very unpleasant to use. Common solvents are phenol, lower alcohols, esters, acetic acid.

The paper must be pure with no sizing, and of even texture. The texture of ordinary filter-paper is often uneven. Whatman No. 1 chromatography paper is suitable for most requirements.

Paper chromatography is essentially a micro-method. Applying too concentrated a solution results in streaks and blobs instead of discrete spots. 60 microgrammes of each component is maximum; thus not more than 600 microgrammes of a mixture of ten amino-acids should be used. Sugars can be applied in larger quantities. The lower limit depends entirely on the sensitivity of the final staining process, e.g., ninhydrin can detect as little as one microgramme. The solution is put on the paper with a capillary tube, micro-pipette, Aglar syringe or calibrated platinum loop. The wet area of the spot must be kept smaller than 1.5 cm. diameter. Control this by immediate drying with warm air. If the solution is too weak, apply another spot on top of the first after it has dried.

With every run it is essential to run standards at the same time in the same tank, e.g., make a solution of 4 sugars that might be found in urine. Rule a pencil line across a paper 4 centimeters from one end. Mark the line at 2 centimeter intervals leaving a 2 centimeter gap at the least from both sides. On each mark, spot separately first the mixture, then each of its components, then the urine under investigation. Sugars separate best by descending technique with a solvent mixture such as butanol-pyridine-water. After running, drying and staining with e.g. aniline phthalate compare the placings and colours of the spots derived from the mixture with each of the individually spotted sugars. Those that correspond in colour reactions and in distance travelled from the starting line are the same. Similarly the spots derived from the urine can be identified. After deciding the running order of the components of the standard mixture it is not necessary to run them separately again, as in the same solvent they always run in the same order.

In a hospital laboratory we are interested mainly in amino-acids, sugars, indole derivatives in urine, and amino-acids in plasma.

Indoles and their derivatives are important in the following:—phenylketonuria, characterised chemically by excretion of phenylpyruvic acid and indolyllactic acid and high plasma concentration of phenylalanine. In metastasising carcinoid there is often a greatly increased excretion of 5-hydroxy-indole-acetic acid or 5-hydroxy-tryptamine. Hartnup's disease is characterised by the excretion of indoxyl sulphate, indolyl-acetic acid, and unidentified indole called U_1, and trytophane. A drop of urine run alongside standard indole spots, then stained with Ehrlich's can decide the presence of these within sixteen hours.

For amino-acid chromatography it is essential to remove proteins and salts from the specimen as their presence causes streaking and blurring. Some workers advise it for sugar chromatograms. It should not be done for indoles. There are three ways of desalting and deproteinizing specimens:—solvent extraction, not easy; electrolyltic desalting, not satisfactory; ion-exchange resins, good. 5 ml. of urine are put on a column of Zeocarb 225 and the amino-acids washed off with ammonia. The eluate is concentrated and the equivalent of 50 microlitres of urine applied to the paper and run overnight in 80% phenol with cupron and ammonia to prevent auto-oxidation of the phenol. The paper is dried without much heat, dipped in ninhydrin, 0.2% in acetone, then heated for several minutes at 100°C. Amino-acid spots stain pinkish.

A clear cut separation is often not achieved and the spots overlap each other. A 2-way chromatogram is then necessary. The urine spot is applied near one corner of an 11-inch square paper which is rolled and sewn with white cotton into a cylinder which will stand on the tank floor. A similar paper cylinder is prepared for the standard spot; each spot needs a paper to itself and the standard must be run in the same tank at the same time as the unknown. After running overnight the papers are taken out, dried and re-stitched in a roll at right-angles to the first direction, and run in a different solvent such as butanol-acetic acid. Sometimes peptides are present. These stain with ninhydrin like amino-acids. To prove their presence gently hydrolyse the urine and re-run, to compare the chromatograms of before and after hydrolysis. Peptide spots should disappear and one or more extra amino-acids appear.

Normal amino-acid excretion is expressed in terms of amino-nitrogen and ranges from 0.2-0.5 grams per day. The main ones are glycine, alanine, histidine, tyrosine and leucine. Excess excretion occurs in liver failure particularly acute yellow atrophy. Generalised amino-aciduria is a primary defect in Wilson's, Fanconi's and Hartnup's diseases. Hartnup's is distinguished by normal proline excretion. In Wilson's disease there are excessive

amounts of particular peptides which on hydrolysis give glutamic acid. Fanconi's syndrome has a non-specific peptiduria and glycosuria as well as amino-aciduria involving about 10 amino-acids. Cystinuria gives an excessive excretion of cystine, lysine and arginine. Lead poisoning has given excess alanine and b-amino-isobutyric acid. Mercury poisoning has given increased excretion of glycine, and poisoning from uranium and cadmium a much greater output of threonine and serine. Amino-aciduria occurs in familial galactosaemia as long as lactose remains in the diet.

Various quantitative methods exist but for most purposes it is sufficient to compare by eye the size and colour intensity of the unknown spot with the standard spot.

Paper chromatography is used for the more complicated separations and identification of steroids, catechols, alkaloids, porphyrins and some enzymes. It has its applications in microbiology. Different species of bacteria on the same medium use and produce different amino-acids, and their metabolism has been the subject of intensive investigation with paper chromatography. It has been suggested that we have here a new and reliable tool for classification of bacteria. Paper chromatography is used in the control of media used in toxin production and in the control and standardisation of the production of antibiotics. The outstanding characteristic of biological reactions is biological specificity which is partly explained by slight differences in chemical constitution. Undoubtedly paper chromatography will play a notable part in explaining this.

There are several good text books and numerous papers published on the subject. The following are a selected few:—

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(7) "Urinary Sugars, Normal, and in Renal and Hepatic Disease", Apthorp (1957), J. clin. Path., **10,** 84.

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SULPHONAMIDE SENSITIVITY TESTS WITH SPECIAL REFERENCE TO THE STERILIZATION OF HORSE BLOOD

H. C. W. SHOTT

(Department of Microbiology, Medical School, Dunedin)

Many routine laboratories are faced with the problem of acquiring an adequate supply of sterile horse blood when contemplating a service for sulphonamide sensitivity testing. Considering the practical difficulties in obtaining uncontaminated blood, ethylene oxide has been put to good use as a means of sterilization. (1).

Set out below is a routine method for sulphonamide sensitivity testing together with the means by which we in this laboratory have provided an ample stock of sterile lysed horse blood.

INVESTIGATION

A poured plate method is employed. Lysed horse blood is added to the plates containing sulphadiazine to annul the effect of sulphonamide inhibitors in the medium (2). From our observations we found that lysed human and/or sheep red cells were not a satisfactory substitute for those from the horse. Furthermore, whereas we originally thought it necessary to use plates having concentrations of 2.5, 10 and 20 milligrams per centum of sulphadiazine, we concluded that for routine purposes one plate containing 2.5 mgm % was sufficient to give satisfactory and reliable results. Growth occurring on these plates after 18 hours' incubation at 37°C are considered resistant strains.

MATERIAL AND METHOD

Lysed Horse Blood

Approximately 2 litres of blood were taken from the animal when slaughtered and received into a sterile bucket containing 200 ml of 3.8% sodium citrate as an anti-coagulant.

After sedimentation the plasma was removed from the red cells and replaced with an equal volume of sterile distilled water. Mixing ensured complete lysis of the cells. The mixture was then transferred to a sterile vacuum flask and placed in the 4°C cold room for 1 hour. After this time 10 ml of chilled liquid ethylene oxide was added by means of a chilled sterile syringe and the flask agitated to thoroughly mix the two components. The flask remained in the cold room for an additional hour and was then incubated at 37°C for a period of 24 hours. The vaporised ethylene oxide was evacuated by vacuum and the lysed blood finally distributed into 100 ml volumes. Subsequently the blood

was proved sterile and the necessary amount incorporated into sulphonamide medium. Sensitivity tests were put up in parallel with plates containing "untreated" sterile lysed horse blood. The results were identical.

Sulphadiazine Solution

Ampoules are supplied containing 250 mgm per ml. This solution is broken down to a strength of 2.5 mgm per ml and stored at 4°C.

Basal Medium

Difco blood agar base is bottled and sterilized in 94 ml amounts.

Preparation of Plates

The 94 ml of agar is melted and allowed to cool to 55°C, 1 ml of stock sulphadiazine solution (2.5 mgm ml) and 5 ml of lysed horse blood are then added.

One should carefully mix at each stage to ensure an even distribution of both sulphadiazine and blood. The plates are poured, six large petri dishes being used per 100 ml of medium, giving the optimum depth of agar.

Inoculum

One drop (approximately 0.02 ml) of the broth culture incubated for antibiotic sensitivity tests should be added to a 2.5 ml volume of nutrient broth giving an approximate 1 : 125 dilution of the original culture. A loopful of the diluted specimen is then seeded on to a segment of the sulphonamide plate. Incubation is carried out at 37°C for 18-24 hours before the results are read.

DISCUSSION

When introducing an additional method into routine laboratory work one must always consider the labour involved and whether the consistency of results justifies both time and expense.

With due regard to technique, the preparation of a batch of plates can be carried out fortnightly. The medium will store several weeks at 4°C without loss of potency. The plate method is economical, eight test strains may be inoculated on to segments of one large petri dish. Reliable results can be maintained if a control plate containing no sulphonamide is included every time the medium is prepared and also special attention paid to the correct amount of inoculum used.

It is apparent that no claim for originality can be made in

presenting a technique for the preparation of sulphonamide sensitivity plates; nevertheless, the method of obtaining a supply of sterile horse blood may prove helpful. If one should choose to use ethylene oxide as a reagent it may be worth while to consider some of its properties. The liquid vaporises at 10.7°C but is a toxic substance which should be handled with due care.

SUMMARY

1. A plate method of sulphonamide sensitivity testing, using lysed horse blood, has been described.
2. It is of particular interest that the sterility of lysed horse blood can be ensured by the use of ethylene oxide.
3. Evidence is put forward that the efficiency of the lysed cells is not impaired through treatment with ethylene oxide.

ACKNOWLEDGEMENTS

I am indebted to Dr N. P. Markham for his help and advice in carrying out this work. I would thank Professor J. A. R. Miles for his permission to make this publication.

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A PASTEURELLA SEPTICA INFECTION FROM A CAT BITE

J. C. MANN
(Pathology Department, Palmerston North Hospital)

GENERAL

Pasteurella septica (P. multocida) is a small gram-negative, non-motile, bipolar-staining cocco-bacillus belonging to the Parvobacteriaceae group. This organism causes haemorrhagic septicaemia in many animals, and was formerly given various names depending upon which animal was involved. Thus, we have had Pasteurella aviseptica, Pasteurella bovis septica, and others. Recent bacteriological studies have shown these strains to be practically identical and the common name has been adopted for them all. The organism appears to be a commensal in the upper respiratory tract of the cat, dog and rat.

Infections in humans consist principally of two forms, one a systemic form with no known portal of entry, and the other a localised form resulting from animal bites. A third and rare meningitic form has been noted, following trauma to the face and head, and suggests that this organism is a commensal in the nasal cavities. The systemic infection may follow a wide range of conditions including bronchiectasis, sinusitis, meningitis, brain abscess, empyema and fulminating septicaemia. The first fully reported case of human systemic infection was in 1919 during the influenza pandemic. Prior to 1947 this disease appeared to be confined to Europe and Asia, but since then 45 cases have been reported in the United States of America.

Localised infection following an animal bite was first reported by Kapel in 1930, and since then approximately 30 cases have been reported. The cat is the most frequent offender, but other animals involved have included the dog, rat, rabbit and panther. The pattern of infection following the bite is that of a localised pyogenic reaction. The area soon becomes painful, red, tender and markedly swollen, with a lymphangitis and lymphadenitis. If no antibiotics are used in treatment, an abscess almost invariably develops which heals slowly over a period of weeks or months. Osteomyelitis frequently occurs, and it has been considered that this organism may have a propensity for bone involvement. However, as no bony involvement has occurred in cases other than cat bite infections, it seems likely that the organism is probably placed directly on the surface or in the substance of the bone by the sharp, needle-like fangs of this animal, as opposed to the blunter fangs of other animals.

The formation of antibodies may reach a high level in sys-

temic infection due to P. septica, but is not common in infections due to animal bites.

CASE HISTORY

The following is a report of an infection due to P. septica which was treated at the Palmerston North Public Hospital during 1957. The patient was first seen at the outpatients' department on the 3rd of September. She had been bitten by her cat, the previous day, on the thumb of the left hand. The thumb was swollen, discharging and very tender. Treatment was commenced with magnesium sulphate and antiphlogistine which was repeated daily, but no improvement in the condition of the wound was noted. On September 7th a swab was taken and sent to the laboratory.

After receiving a preliminary report, the casualty department commenced treatment of the patient with intramuscular penicillin on September 10th, daily injections being given until September 18th. An x-ray taken 14 days after the onset of the infection showed no signs of bone involvement. x-ray therapy commenced on September 19th and a slight improvement in the condition of the thumb was noticed. At the end of September, after a course of x-day therapy, the wound was almost healed, although the thumb was still very swollen. A further x-ray of the thumb taken on October 11th showed slight changes of the bone due to the infection, but no signs of osteomyelitis.

At this stage the lesions were completely healed, but the thumb was still very painful and swollen, and the patient was unable to move it at all. After a fortnight's treatment at the physiotherapy department, there was very little improvement and no further treatment was attempted. When seen by the writer six months from time of infection, the thumb was normal in appearance though movement was still very limited.

LABORATORY INVESTIGATIONS

The culture on blood agar showed a pure growth of an organism with a small, round, opaque, greyish-coloured colony, showing a slight amount of alpha-haemolysis, which disappeared on subsequent cultures. The organism would not grow on MacConkey agar, but broth cultures were readily obtained. Tests showed it to be sensitive to penicillin, streptomycin, tetracycline, chloromycetin and terramycin.

On staining, the organism appeared as a pleomorphic gram-negative bacillus with a marked tendency towards coccal forms, especially when grown on a solid medium. When stained with methylene blue the bacilli tended to show bipolar staining though

this was more readily obtained when grown in liquid cultures.

The organism was tested for biochemical reactions and it was found to produce acid, without gas, in glucose, mannitol sucrose, galactose, xylose and sorbitol, while no reaction was observed in lactose, maltose, salicin, dulcitol, rhamnose, raffinose, arabinose, inulin, inositol or trehalose. Indole was produced, methylene blue reduction was positive, methyl red and Voges-Proskauer tests were both negative, while no change was observed in litmus milk.

Mouth swabs were taken from the offending animal and a pure growth of a similar organism, giving the same biochemical reactions, was obtained. A culture of Pasteurella septica, obtained from a pig, was procured, through the National Health Institute, for comparison.

0.2 ml of broth culture of the organism was injected intraperitoneally into mice to test for pathogenicity, two mice being injected with the organism from the wound, two with the organism from the cat's mouth, and two with the stock strain of P. septica. The mice injected with the wound organism both died eight days later, while one injected with the culture from the cat's mouth died after 48 hours and the other after ten days. Of the mice injected with the stock strain of P. septica one died after ten days and the other was still alive after 16 days. The organism was recovered from the heart blood of the first four mice which died, but was not recovered from the mouse which died ten days after injection with the stock strain of P. septica, while the other mouse injected showed no ill effects. These results could possibly be explained by the fact that overseas tests show that this organism loses much of its pathogenicity after prolonged artificial culture.

Blood specimens were obtained from the patient at periods of three weeks and two months after the onset of the infection, but no specific antibody formation was demonstrated. Although the patient's serum agglutinated all three cultures of P. septica to varying titres, the same or higher titre aggultinations resulted from using other presumably normal sera.

ACKNOWLEDGEMENTS

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STAFFING PROBLEMS IN DISTRICT LABORATORIES

M. JENNER
(Bacteriology Department, Hawera Hospital)

It is now some time since Mr Scott's paper on the problems of the smaller laboratory, but the situation concerning staff has deteriorated rather than improved, and it seems that the time has come for some reconsidcration of the regulations relating to training.

From the purely abstract viewpoint, it is evident that the people who live in the country districts and in the smaller towns are entitled, as taxpayers, to an efficient laboratory service, even if it is not as comprehensive as that enjoyed by city-dwellers.

Our statistics show that district laboratories, in common with the base laboratories, have had a very considerable increase in the amount of work demanded of them in the last few years. This increase includes a greater range of tests as well as a greater number, and in order to deal with them adequately with a small staff an efficient method of handling is necessary. This efficiency can be maintained only when a reasonable proportion of skilled personnel is available, and that is our greatest problem.

The district laboratories have long since graduated from the stage where they did blood and urine examinations and handed everything else on to the base laboratories. In my own laboratory we send away sera for W.R. and complement fixation tests, animal inoculations, Rh genotyping, specialist biochemistry, and, of course, histology, but we do not send away tests which can be technically and economically undertaken here. This would apply to most of the larger district laboratories, and I suggest on this basis, that a much larger proportion of the five year training period could be spent by the trainees in their "home" laboratories. Allowing 12 months as an adequate time for senior trainees to spend in a base hospital gaining experience in those branches not available to them in the district laboratories, I suggest that the training regulations could now reasonably be amended to allow trainees to spend another year in their original laboratories before transferring to a base hospital to complete their training. This would enable the base laboratories to fulfil their obligation to train, without leaving the smaller laboratories in their present state where senior staff are forced to leave at a time when they have become really useful members of their establishments.

It is most disillusioning to have a continual stream of very junior trainees starting from school, passing through first year when they absorb more time in training than their presence saves in work, through second year when they are more useful but

still require a good deal of supervision, to third year when they are more versatile and useful, and then to lose them inevitably because of training requirements. From the point of view of the base laboratories it must be most satisfactory to have this continual harvest of partly-trained personnel, but from the viewpoint of those of us who have to organise the district laboratories, it makes one wonder whether trainees are worthwhile when, like the daffodils, they "haste away so soon".

In country districts, there is a definite shortage of young people whose educational background and personality makes them suitable for acceptance as trainees. May I make a plea that we be allowed to retain for another year those trainees we do manage to find from within our own districts, and who are willing to stay if they are permitted to do so.

There seem to be two ways in which the shortage of senior staff can be alleviated in the district laboratories. One alternative would be for the base hospitals to undertake all training, staffing the district laboratories only with trained personnel, technicians, and cleaners. This would probably give greater continuity and, within the limited scope of the technicians, greater working efficiency. It would have the less satisfactory effect of forcing potential trainees to leave home for the whole of their training period.

The other alternative, and I offer it with some trepidation, is a "country-service" requirement in the regulations for personnel who wish to attain the status of department heads or charge positions. It could provide for six months in a district laboratory immediately after qualifying as a prerequisite to grading beyond Scale "D", and would provide much help for the officers in charge of the smaller laboratories, acting as some sort of compensation for the staff who are required to move to base laboratories. At the same time it would provide the attached senior staff with some insight into the particular problems of the smaller units. It seems fundamentally unsound that bacteriologists can reach very senior status without ever having served outside the walls of their original laboratories.

This latter scheme would take care of the ever-present problem of obtaining relievers for annual leave. At present, neither the Department nor all the base laboratories supply relievers. It would be most helpful if the Health Department, or some other responsible body, employed one or two qualified bacteriologists on, say, a 12 month contract, to act as relievers for laboratories all over the country where no qualified bacteriologist is available to take charge in the absence of the senior person. An inducement such as reasonable accommodation expenses, and a temporary grading, would combine with the intrinsic interest of such a

temporary appointment to ensure that one or two qualified people were always available.

In the seven years that I have been in charge of my present laboratory, I have only once been able to leave it in charge of a qualified person. On every other occasion it has had to be left in the charge of a two or three-year trainee. This is not fair to the trainee concerned, and it is certainly not in the best interests of the people of the district. No matter how competent the trainee may be, there is no substitute for practical experience, especially in emergency procedures.

It is not sufficient merely to dismiss the problem as not affecting one's own laboratory. Our profession, like any other, has a joint responsibility to the public, and the present system of staffing the district laboratories does not enable us to maintain the service at a uniformly high standard.

Until some provision is made to give some relief, and adequate staff is available at all stages of training, those of us who are charged with the administration of smaller laboratories will continue to accumulate leave which resembles the National Debt in its propensity to increase with the passage of time and never to be reduced.

TO SUMMARIZE, it is suggested that:—

1. Trainees be allowed to spend an extra year after intermediate in their "home" laboratories if they wish to do so.

2. A "country-service" clause be incorporated in the training regulations governing advancement to senior positions.

3. A permanent staff of qualified relievers be set up and made available to laboratories throughout New Zealand.

UROPEPSINOGEN ESTIMATION AS A FURTHER LABORATORY AID IN THE DIAGNOSIS OF PERNICIOUS ANAEMIA WITH AN ILLUSTRATIVE CASE

R. KENNEDY

(Haematology Department, Auckland Central Laboratory)

It has been found that the excretion of uropepsinogen in cases of pernicious anaemia is very low. Goodman, Sandoval, Halstead (1952) used a method whereby the uropepsinogen is converted to pepsin and the enzyme digests a buffered casein substrate. The results are reported in units excreted per hour. The normal range is 15-40 units.

This test has been in use at this laboratory for some months now and has been found to be of special value in excluding pernicious anaemia, particularly in cases where liver extracts have been administered prior to laboratory investigations.

Case History

An elderly woman was admitted to hospital with a history of gastro-intestinal symptoms, nausea, loss of appetite, shortness of breath and dizziness.

Laboratory Investigations

Haemoglobin	6.0 gm./100ml.
Red cells	2,250,000/cu. mm.
Colour index	0.95
Haematocrit	20%
MCV	90 cu. microns
MCHC	30%
ESR (Westergren)	81 mm.
White cells	5,000/cu. mm.
Differential	
Polymorphs	67%
Eosinophils	3%
Lymphocytes	27%
Monocytes	3%

Red cells showed anisocytosis, macrocytosis and microcytosis.

Reticulocytes	1.4%
Platelets	109,000/cu. mm.
Coombs	negative
Alkali denaturation	no foetal haemoglobin detected

Haemoglobin electrophoresis, normal adult pattern.

None of the routine laboratory tests to this point would provide the clinician with a positive diagnosis but the following two results would confirm the provisional diagnosis of pernicious anaemia.

Uropepsinogen 4.2 units/hr (normal 15-40)

A bone marrow biopsy was found to be classically megaloblastic. Vitamin B_{12} therapy was commenced and a reticulocyte "crisis" was reached within a week.

DISCUSSION

Diagnosis of classical pernicious anaemia is made more difficult these days by the administration to patients of liver extracts prior to routine laboratory investigations. The uropepsinogen estimation while not of great value alone in diagnosing the condition is of considerable use in excluding pernicious anaemia. The method is relatively simple and can be performed routinely by most laboratories.

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TRANSFUSION SERVICES IN SMALLER HOSPITALS

M. JENNER
(Bacteriology Department, Hawera Hospital)

In March of this year a survey of the smaller blood banks in New Zealand was undertaken. Seventeen circulars were sent out with questionnaires to bacteriologists in charge, and 12 were returned completed.

The purpose of the survey was to try to establish what the standard practice is in these blood banks, away from the main centres. This was thought to be worthwhile, because these banks have grown as the demand for blood has increased in their respective districts. There has been little or no co-ordination in this growth of the individual banks, and their solutions to their own local problems are naturally of considerable interest to officers in charge of other banks, as well as to bacteriologists and pathologists in general.

There was no intention to suggest which customs are the best, or to offer criticism, but purely a desire to produce an over-all picture of existing practice.

The questions set out on the survey sheet were as follows:—

1. Total number of donors.
2. Normal bank stock.
3. Do you do a haemoglobin check on donors when they give blood?
4. Do you make plasma from expired blood?
5. Do you send it away for processing?
6. Do you provide iron in any form for your donors?
7. How often do you call donors in these groups:—
   (a) O. Rh positive.
   (b) O. Rh. negative.
   (c) A. Rh. positive.
   (d) A. Rh. negative.
   (e) B. Rh. positive.
   (f) B. Rh. negative.
8. Do you provide transport for donors?
9. What form does it take?
10. Do you send an acknowledgment to donors showing the nature of the case for which their blood was used?
11. How often do you encounter employer reluctance to allow donors off work to give blood?
12. Do you have a regular day for bleeding donors?
13. Are donors bled in (1) operating theatre, (2) outpatients department, (3) laboratory.
14. Are masks worn by (1) operator, (2) assistant, (3) donor.

15. How long are donors at the hospital including time for refreshments?
16. What stimulants do you offer?
17. Who does the actual bleeding of donors?
18. Are "bleeding trips" undertaken away from the hospital?
19. Are disposable collecting sets used?

QUESTION

1. *Donors' Roll:* This varied from 80 to 760. They were distributed as follows:—80, 135, 154, 192, 260, 320, 350, 410, 500, 540, 600, 750, 760.

2. *Bank Stock:* Three of the smaller laboratories carried no stock—calling donors as required. The range of the others was 1, 5, 6, 8, 10, 12, 14, 16, 20, pints average stock.

3. *Haemoglobin Tests:* Six banks carry out routine checks although one does them on women only.

4 and 5. *Plasma:* None of the banks in this group make plasma or send it away for processing.

6. *Iron:* Two of the groups provide iron for donors.

7. *Frequency of Calling:*
   O. Rh. positive 4 months to 2 years.
   O. Rh. negative 3 months to 1 year.
   A. Rh. positive 4 months to 3 years.
   A. Rh. negative 4 months to 2 years.
   B. Rh. positive as required.
   B. Rh. negative as required.

8 and 9. *Transport:* With two exceptions, transport is provided for donors. This varies considerably. Eleven provide taxis as their normal procedure. One pays 3/6 towards petrol as an alternative, one bacteriologist uses his own car on a mileage allowance. One hospital uses a car provided by the St. John organisation, and driven by voluntary drivers.

10. *Acknowledgments:* Six laboratories send out acknowledgments although three of these do not indicate the nature of the case.

11. *Employer Reluctance:* This was not reported in any case.

12. *Regular Day:* Only three laboratories stated that they had a regular day. The remainder bled donors as required.

13. *Where Bled:* Six hospitals bleed donors in the outpatients department. Five of the smaller ones use the operating theatre (only one of these normally carried six pints or more), two have a suitable room in the laboratory.

14. *Use of Masks:* In five cases the operator wears a mask. In one case all present, *including the donor,* are masked. Eight

hospitals do not use masks at all when bleeding donors but concentrate upon skin preparation.

15. *Total Time Involved:* Three banks keep their donors only 15-20 minutes. Seven average 30 minutes, and two take about 45-50 minutes.

16. *Stimulants:* There was considerable variation in the range of stimulants offered. One offers tea or cordial. The others offer tea with brandy, whisky, beer, or stout as alternatives. In general, 50% of donors request alcoholic stimulants although some ask for a nip of spirits followed by a cup of tea.

17. *Who Bleeds Donors:* In eight hospitals technical staff bleed donors although in four cases this refers only to qualified bacteriologists. In four hospitals the house surgeons bleed donors, and in one case medical officers and technical staff share this duty.

18. *Outside Bleeding of Donors:* Only two banks in the survey regularly carry out bleeding of donors away from their own hospital. One other does so occasionally.

19. *Disposable Sets:* Only one hospital in the survey does not use disposable sets.

It is interesting to reflect upon what proportion of the population of the respective hospital board's areas are blood donors in each case. This figure varies from 0.39% to 4.0% with an average of 1.77%.

Taken over the whole country, a considerable volume of plasma is available from the laboratories covered in this survey, if an expedient means of separating it and transporting it could be found, and if the base laboratories became interested in acquiring it. The process of preparing even small pool plasma is outside the scope of district laboratories, because of shortage of staff, special equipment and, in most cases, a suitable room.

The fact that only six laboratories actually send acknowledgments to their donors shows that this is a factor affected by local custom. The writer has often been told by donors that they like to receive these acknowledgments, and in this district they certainly expect them and are interested in them. The sending out of these notes requires a considerable amount of time, but in South Taranaki the goodwill involved undoubtedly justifies the effort.

Most small banks, having evolved slowly from donor panels where no stock was carried, began by using the operating theatre as a place to bleed donors. As they have grown this custom has become less satisfactory because bank needs and theatre availability clash too frequently. The next stage of development, the use of outpatients department, is more flexible and, as disposable

sets are now standard practice, neither the operating theatre, nor its staff, needs to be involved in the bleeding of donors, who can then be bled whenever it is expedient and the donors are available.

From a psychological viewpoint it would seem that it is better to regard the giving of blood as a semi-social occasion as far as the donors are concerned. For this reason, it is probably unsound psychologically to take donors into the operating theatre, let alone bleed them on the operating table in an atmosphere associated with "the drama of medicine" in the lay mind. For the same reason, the use of masks is probably undesirable, especially when so much can be achieved by adequate skin preparation.

In this area and probably in all others of comparable size, there are many donors in the adjoining country areas who would give blood if they did not have to make a special journey to town after the evening's milking or other farm work. When the bank stock is only 6-8 pints, it is questionable whether a trip of 10-15 miles into the country to bleed so few donors is worthwhile. In our case there are four or five country centres where an evening's bleeding would fill the bank, and relieve the strain on the town donors. For this reason, it is intended to make some experimental trips to decide whether the yield justifies the time and travelling involved.

Since the introduction of disposable collecting sets, which this survey shows to have been very widely adopted, a great deal of time and handling is saved. Their obvious advantage for bleeding trips are also evident, especially their compact nature and small bulk. On first examination, their price (about 6/6) seems relatively expensive, but a strict accounting of the cost of time spent in handling, cleaning, checking, sharpening and assembling the conventional sets, makes even this first impression of the cost seem doubtful. Even if they are, in fact, slightly more expensive, it cannot be suggested that 6/6 is an unduly high price to pay for a pint of blood, when the equipment used in its collection is pyrogen free and needs no maintenance.

MINUTES OF A COUNCIL MEETING OF THE N.Z.A.B.

Held at Wellington Hospital on Saturday, 12th April, 1958

1. *Present:* Messrs Reynolds, Olive, Murphy, Donnell, Walsh, Bloore, Tanner and Miss Evans.
2. *Apology:* Mr Corey.
3. *Minutes of Previous Meeting:*
   Moved: That these be taken as read. Olive—Bloore.
4. *Business Arising from Minutes:*
   Moved: That the minutes as published be adopted. Murphy—Walsh.
5. *Applications for Membership:*
   The following new members were elected:
   Miss A. Slee (Nelson), Miss A. Foley (Christchurch), Miss V. Tucker (Christchurch), Miss R. J. McLeay (Auckland). Olive—Bloore.
6. *Resignations:*
   The following resignation was accepted with regret:
   Mr E. R. Smith. Tanner—Bloore.
   A letter was received from Mr F. Corey tendering his resignation from the Council. The President expressed his regret at Mr Corey's retirement.
   Moved: That the Hon. Secretary be instructed to write to Mr Corey accepting his resignation with regret. Olive—Bloore.
7. *Financial Report:*
   The Treasurer reported on the finances of the Association and urged members to assist in expediting the payment of subs. now due.
   Moved: That the Treasurer be authorised to pay members' expenses to the Council Meeting. Olive—Reynolds.
8. *Journal Report:*
   Miss Evans, the Editor, reported that the present supply of articles if it continued, was satisfactory. Mr Rose, her Co-editor, was ill, which had increased work somewhat although he was still able to help with proof reading, etc. The President thanked Miss Evans for the report and expressed gratification at the current stability of affairs.
9. *Correspondence:*
   Letter from Mr J. D. Morgan asking for assistance in salary negotiations. The President explained that the Association was unable to help directly and outlined some of the difficulties. The Secretary was instructed to write to Mr Morgan amplifying the position from the Association's view.
   Letter from Mr J. J. Cannon asking permission to represent the Association at discussions overseas.
   Moved: That the Hon. Secretary write to the Secretary, I.M.L.T., Great Britain, asking that Mr Cannon be acknowledged as representing the N.Z. Association of Bacteriologists (Inc.). Walsh—Olive.
   Sundry Departmental Notices and Routine Letters.
   The President explained the action he had taken in writing to the Society of Pathologists.
   Moved: That the action taken in writing to the Society of Pathologists be approved. Bloore—Walsh.
   Moved: That inward correspondence be accepted. Olive—Murphy.
   Moved: That outward correspondence be approved. Murphy—Walsh.
10. *General Business:*
    (a) *Library Proposal:* A letter was received from Mrs Corey expressing her willingness to proceed with the work of Librarian and outlining several proposals. After a discussion members thought it

redundant to establish an additional library to the facilities already available. It was thought that a system should be devised whereby Association members would make use of the various libraries through the Association librarian.

Mr Reynolds was instructed to enquire about facilities at the National Library Service and Health Department Library.

The Hon. Secretary was instructed to write to the Canterbury Medical Library similarly and to write thanking Mrs Corey for her continued offer to act as Librarian and to tell her of the Council's proposals.

Moved: That the Treasurer be instructed to send Mrs Corey the sum of £5:0:0 (five pounds) to cover interim expenses. Olive—Bloore.

(b) *Addition to Rules:* To allow formation of branches.

The President referred members to the rules of similar incorporated societies on the forming of branches and members discussed points arising from these as they affected the Association. Mr Murphy outlined his thoughts on the subject.

Moved: That Mr Reynolds and Mr Olive be appointed a sub-committee, with power to act, to make provision for addition to the rules of the N.Z. Association of Bacteriologists (Inc.) to allow the formation of local branches. Murphy—Donnell.

The sub-committee was instructed to proceed with this matter as outlined in the discussion by members.

The meeting closed at 1.15 p.m.

EMPLOYMENT REQUIRED

A letter has been received from an English laboratory technician who holds the F.I.M.L.T. in haematology and pathological technique, seeking a position in a New Zealand hospital laboratory. Haematology and serology are her main interests.

Address: Miss R. A. Holemans, 83 London Road, Kettering, Northants, England.

EMPLOYMENT OFFERED

Notice of a vacancy has been received from the University of London, Postgraduate School of Medicine, offering a technician's position in their Experimental Surgical Laboratories to someone with biochemical knowledge. Further information can be obtained from the Hon. Secretary, N.Z. Association of Bacteriologists (Inc.).

EXAMINATION FOR CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

FEBRUARY, 1958

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Dr. J. D. Reid, Dr. N. U. C. Godfrey, Dr. N. G. Prentice.

WRITTEN PAPER

Tuesday, 25th February, 1958, 9.30-12.30 p.m.

All questions are to be answered. All questions carry equal marks.

1. Discuss the methods commonly employed for the estimation of proteins in blood.
2. Write short notes on:
   1. The principles of flame photometry.
   2. Reducing substances in urine.
   3. Estimation of glucose of C.S.F.
   4. The standardization of solutions of sodium hydroxide.
3. Discuss in detail two methods (preferably the one you use and one other good method) for the routine estimation of blood haemoglobin. What checks do you advise to see that your results are accurate?
4. Give the normal blood findings (including expected variations) in a "full blood-count" for a healthy full-term infant at birth. Describe the appearances of the baby's film.
5. How would you distinguish pathogenic from non-pathogenic members of the following groups.
   1. Mycobacteria.
   2. Staphylococci.
   3. Streptococci.
6. Write brief notes on the practical use of–
   1. Selective media.
   2. Anaerobic culture.
   3. Complement.

PRACTICAL I

Tuesday, 25th February, 1958, 2.30-5.30 p.m.

BIOCHEMISTRY I

1. Estimate the serum calcium level in the specimen (A) supplied.
2. Write notes on the set out reagents and equipment indicating method of use.
   (Nessler's reagent, van Slyke apparatus.)

BACTERIOLOGY I

1. Examine the throat swabs obtained from a child with a greyish exudate over the tonsils.
   (C. diphtheriae.)
2. Identify culture A.
   (Flexner W.)
3. Specimen B. is the suspension of an organism isolated from C.S.F. Identify this.
   (Staphylococci and H. influenzae.)
4. Identify cultures C. and D.
   ("C" sporing motile bacillus. "D" Staphylococcus aureus.)

Questions 1, 2, 3 and 4 may be completed on Wednesday morning.

5. Identify cultures E and F.
   ("E" Haemolytic streptococcus. "F" Pseudomonas aeruginosa.)

PRACTICAL II

Wednesday, 26th February, 1958, 9.30 a.m.-12.30 p.m.

BACTERIOLOGY II

1. Complete questions 1, 2, 3 and 4 from previous day.
2. Identify as far as possible G, H and J.
   ("G" Saccharomyces, "H" Cl. welchii, "J" Brucella.)

HAEMATOLOGY I

1. Estimate the P.C.V., R.B.C., Hb., W.B.C. and differential and work out the indices on the given sample of blood.
   Make a film and report on it.
   (Normal blood.)
2. Stain the given bone marrow film with:
   (a) Jenner Giemsa, or
   (b) May Grunwald Giemsa stain.
3. Estimate the "prothrombin time" on the given plasma and on the control plasma.
   Write a short note on the method you would use to make the thromboplastin solution.

PRACTICAL III

Wednesday, 26th February, 1958, 2.30-5.30 p.m.

BIOCHEMISTRY II

1. Test the urine specimen supplied for sugar, ketone bodies and bilirubin.
   (Sugar—nil, ketone bodies and bilirubin present.)
2. (a) What is the abnormal haemoglobin derivative in the blood supplied.
   (Carbon monoxide.)
   (b) The previous sample of blood contains 600 mgm. per 100 ml. of NaCl. Convert this to milli-equivalents per litre. (Na.23. Cl.35.5.)
   (c) Examine the specimen of faeces for occult blood.

HAEMATOLOGY II

1. Estimate the platelet count on the given sample of blood.
2. Stain and report on the film from a woman aged 58 years. The count shows 6 gm. of Hb. and a W.B.C. of 5400.
   (Atypical lymphocytes.)
3. Do a direct Coombs' test on the given blood.
   (Coombs' test—positive.)
4. Write short notes on the use of the two given pieces of equipment.

ORAL EXAMINATION

Subjects discussed at the Oral Examination were:—

Dr. Reid: Leptospira, pathogenic E. coli, bacteriophages, soluble haemolysins, antistreptolysin titres, antibodies, Black Leg, pulpy kidney, Johne's bacillus, Brucella types, Z.N. tissue stain.

Dr. Godfrey: Red cell fragility, immature white cells defibrinating apparatus, preparation and staining of blood films, appearances of red cells in blood disorders, haemoglobin determinations, cross-matching, L.E. cells.

Dr. Prentice: Photoelectric colorimeters, faecal fats, buffers for thymol turbidity tests, liver function tests.

SUCCESSFUL CANDIDATES

Mr L. M. PAUL (Lower Hutt).
Mr E. R. SMITH (Timaru).
Miss D. M. MACLEAN (Wellington).
Mrs R. McDOWALL (nee Saunders) (Dunedin).
Mr J. F. LYON (Wanganui).
Mr P. G. SNOW (Christchurch).
Miss P. A. FURKERT (Auckland).
Miss S. E. WHYTE (Wellington).
Mr G. L. CHAMBERS (Auckland).
Miss R. T. CANTRELL (Palmerston North).
Mr G. KURU (Wanganui).
Mr I. C. T. LYON (Wellington).
Sister M. KILLIAN (Miss Mary McKeever) (Auckland).
Mrs J. B. MONRO (nee Petersen) (Palmerston North).

All candidates who presented themselves for this examination were successful.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

APRIL, 1958

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Dr. W. A. Russell, Mr D. H. Adamson.

THEORETICAL EXAMINATION

Tuesday, 29th April, 1958.

Time allowed: 3 hours.

All questions should be attempted. All questions carry equal marks.

1. Describe in detail the method used in your laboratory for the isolation and identification of an organism of the genus Salmonella from a specimen of faeces.
2. What methods are available for the precipitation of proteins in blood? Describe in detail any one method.
3. (a) Briefly tabulate ten laboratory accidents, some of a personal nature and some which may occur to apparatus. In an adjacent column briefly tabulate the correct procedure for avoiding these "accidents."
   (b) Tabulate the literal meanings of ten words, which are not of Anglo-Saxon origin, used in pathology laboratory terminology.
   (c) Tabulate the steps you would take in preparing a basket of 6in x 5/8in test tubes containing live cultures so that they will be suitable for issuing to the wards for collecting specimens of blood for the Wassermann reaction.
4. Describe the principle and method of blood sugar estimation by either Folin and Wu or King's colorimetric method. Explain the calculation of the result using symbols and assuming the blood sugar is not greater than 200mgm %.
5. (a) Draw a diagram of the ruled area of a Neubauer counting chamber (or improved Neubauer). Indicate the measurements.
   (b) What are the usual dilutions used in performing red and white cell counts? Explain the calculation of the red cell count.
   (c) Write the formulae for the absolute indices and state their normal range of values.
6. (a) Describe concisely your technique for an ordinary routine cross-match (given that the group of your patient is "A").
   (b) Describe concisely your technique for a reticulocyte count, including your method of enumeration. What are reticulocytes and what is their significance?

PRACTICAL EXAMINATION I

Tuesday, 29th April, 1958.

BACTERIOLOGY

Time allowed: 1 hour.

1. (a) Name article A and explain in a few words how it is used.
   (Lovibond comparator.)
   (b) What is article B. Is it in good order?
   (Thermostatic capsule—exploded.)
   (c) State what article C is, and how it is used.
   (Eyepiece micrometer.)
2. The swab D is from an infected leg wound of three days' duration. Report upon a direct film and, as far as possible in the time allowed,

identify the organisms by cultural methods. Sensitivity tests to the four antibiotics provided should be carried out on the primary culture. State what further tests could be carried out to confirm the identity of the organisms.
Time allowed to complete this question on the following morning.
(S. aureus and diphtheroids—Str. haem. did not grow.)

3 Report upon the "spots" under the six microscopes from left to right, from: urethra, spinal fluid, mouth, pleural fluid, blood culture, urine. N. gonorrhoeae, H. influenzae, Vincent's organisms, Myco. tuberculosis Br. abortus, cystine crystals.

1. Make a wet film of the urine deposit, E, and report upon the microscopic findings.
(Pus, blood, crystals, bacteria, casts.)

BIOCHEMISTRY
Time allowed: 1 hour.

1. Estimate the total non-protein nitrogen in the specimen of oxalated blood, F. The standard provided contains 30 mgm. per 100 ml. of nitrogen.
2. Determine the normality of the approximately tenth-normal solution of NaOH provided (M) by titration with standard N/10 HCl.
Show calculation in full and state how one litre of accurate N/10 NaOH would be prepared from this solution.

PRACTICAL EXAMINATION II
Wednesday, 30th April, 1958

HAEMATOLOGY
Time allowed: 1½ hours.

1. (a) Perform a Hb. estimation, red cell count and P.C.V. on the blood sample P. State briefly the essential details of the steps taken.
Calculate the M.C.H. concentration.
Examine the blood film Q, state whether it could have been made from blood P, and give reasons.
(b) Make a film from blood sample S, stain it with Leishmans, label and leave for inspection.
(c) Determine the A.B.O. group of blood samples X, Y and Z and detail the technique briefly.
2. (a) Perform a differential count on blood film W.
(b) Report on the four blood films provided (1-4).
(Differentials not required.)

ORAL EXAMINATION

Subjects discussed at the oral examination were:

Normal values, neisseriae, T.N.P.N., satellitism, identification of P. vulgaris, Cl. welchii, Ps. pyocyanea, C. albicans, salmonellae and shigellae, Ehrlich's reagent, washing tubes, urinary deposits, microscopes, corynebacterium and haemophilus groups, various media, blood sugar, autoclaving, precautions against dirt in the laboratory, protein precipitation, antibiotic sensitivity tests, accidents, postal regulations, graduated pipettes and other measuring equipment, methods of examination for Myco. tuberculosis, occult blood, methods of sterilization, test meals, staphylocoagulase, thermostats, milk and Br. abortus.

SUCCESSFUL CANDIDATES

BROOKS, A. B. (Auckland).
BUCHANAN, M. J. L. (Auckland).
BUTCHER, M. A. (Oamaru).
CARSON, P. D. A. (Wellington).
CATER, J. M. (Wanganui).
CHESTERMAN, H. J. (Drs. Brown and Lusk).
CHURCHOUSE, M. J. (Auckland).
CLARKE, E. B. (Auckland).
CRAVEN, D. M. (Dunedin).
ECCLES, N. J. (Auckland).
FORBES, M. J. (Gisborne).
FORD, A. E. (Dunedin).
HANDLEY, K. C. (Tauranga).
HARPER, P. V. (Greymouth).
HARRISON, P. M. K. (Auckland).
HENRY, D. F. (Hamilton).
HOWELL, A. C. (Hamilton).
JONES, P. A. (Auckland).
KING, I. C. (Auckland).
KIRKUP, L. M. (Auckland).
LEADLEY, M. D. (Gisborne).
LEVIEN, J. P. (Wellington).
LYNCH, M. J. (Wellington).
MOORE, B. L. (Christchurch).
McKENZIE, J. M. (Auckland).
O'SULLIVAN, S. D. (Auckland).
PEARSON, C. J. (Wanganui).
PORTCH, J. A. (Dunedin).
PREBBLE, H. G. (Auckland).
RUTHERFORD, G. A. (New Plymouth).
SCOTT, T. R. G. (Auckland).
SNOOK, S. I. (Auckland).
STENHOUSE, P. M. (Christchurch).
THOMAS, J. C. (Auckland).
WILSON, L. R. (Dunedin).

BOOK REVIEW

CHROMATOGRAPHIC TECHNIQUES—CLINICAL AND BIOCHEMICAL APPLICATIONS

Ivor Smith, B.SC., Ph.D., F.R.I.C.

1st Edition—1958—309 pages. Price 45/- (Eng.)
Wm. Heinmann, Medical Books Ltd., London

This excellent book by a group of London biochemists under the editorship of Ivor Smith presents in a clear practical manner the techniques of paper chromatography. The first four chapters deal with apparatus, general techniques and the desalting of fluids. The remaining chapters are devoted to the chromatography of groups of compounds and the authors state clearly their choice of solvents and location reagents for each group. Aminoacids, indoles, sugars, barbiturates, steroids, purines, organic acids are among the groups covered. Many comprehensive tables showing the RF values of a large number of compounds in different solvents are given. Where the compounds are of medical interest a section on clinical interpretation is included. Very little space, however, is devoted to column chromatography.

Although the book contains a considerable amount of information, it is clearly written, well set out, and the typography is excellent.

This book should prove invaluable both to those commencing paper chromatography and to more experienced biochemists.

J.T.M.

ABSTRACTS

DROPLET METHOD FOR OBSERVATION OF LIVING UNSTAINED BACTERIA

A. S. Michael (1957)
J. Bact. **74,** 831

The method eliminates the necessity of making the somewhat dangerous hanging drop preparation of pathogenic organisms.

A drop of immersion oil is spread over an area of a microscope slide slightly larger than the coverslip. A small loopfull of the culture to be examined is placed on the coverslip and this is inverted on to the oil and pressed down. The preparation can now be examined with any objective either by bright field or dark field illumination.

THE ERYTHROMYCIN GROUP OF ANTIBIOTICS

L. P. Garrod (1957)
Brit. med. J. **2,** 57

The merits of spiramycin, oleandomycin and erythromycin are discussed and the conclusion reached is that neither oleandomycin nor spiramycin has been shown to be therapeutically superior to erythromycin.

The synergic action *in vitro* of a mixture of tetracycline and oleandomycin was not confirmed.

The cross resistance among antibiotics of the erythromycin group was also studied.

RH. NOTATION

A. E. Mourant (1957)
Brit. med. J. **2,** 461

This paper describes the present state of knowledge of the two theories and systems of notation; that of Weiner and of Fisher and Race.