



Vol. 5 - No. 3.

July, 1950

JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

EDITED, PRINTED AND PUBLISHED FOR THE ASSOCIATION

BY

DOUGLAS WHILLANS

CONTENTS

Editorial	45
Staphylocoagulase Ian D. Scott	46
Plasma Viscosity A. Fischman	52
A Modified Somogyi Diastase Method F. M. Hilder	53
Department of Health, Intermediate Examination for Hospital Laboratory Trainees, May, 1950	57
Here and There	59

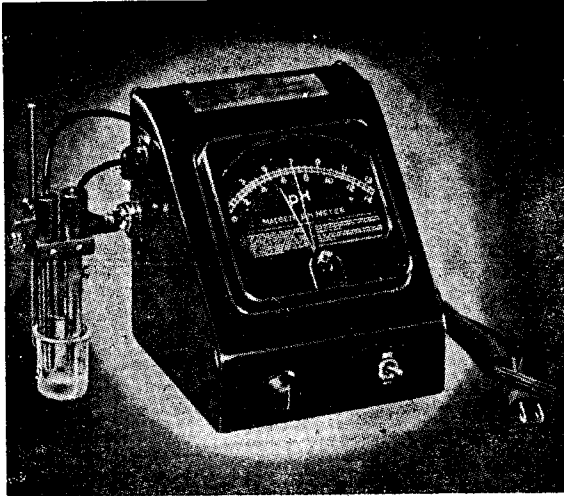
Communications regarding this JOURNAL should be sent to the Editor at the Department of Pathology, Public Hospital, Auckland, C.3.

Communications primarily affecting the Association should be addressed to the Secretary, Mr. G. W. McKinley, Bacteriology Department, District Hospital, Waipukurau.

All monies should be paid to the Treasurer of the New Zealand Association of Bacteriologists (Inc.), Mr. H. T. G. Olive, Pathology Department, Public Hospital, Wellington.

Subscription to this JOURNAL is five shillings per year or one shilling and six pence per copy, post free.

The Accurate Measurement of pH



Fast and accurate pH tests are essential in modern product control. Wherever pH is a factor—in laboratory or industry—Watvic can supply a suitable pH Meter to deliver precise data quickly, accurately and economically.

Illustrated is the MacBeth pH Meter, which operates from line voltage. This accurate industrial instrument is designed to provide such simplicity of operation and freedom from maintenance that it may be used by unskilled workers for routine control.

Also available are the Cambridge Meter, and Instruments for continuous pH recording.

We are pleased to offer our technical services, not only to assist in selecting suitable equipment, but also in its subsequent installation and maintenance. A comprehensive Repair Service is available. Your enquiries on all types of scientific equipment will be welcomed. There is, of course, no obligation.

WATSON VICTOR
LIMITED
(INCORPORATED IN NEW SOUTH WALES)

KELVIN CHAMBERS, 16 THE TERRACE, WELLINGTON.

With Branches at Auckland, Christchurch, Dunedin and all
Australian States.

"SERVICE TO SCIENCE"

WE SUPPLY MICROSCOPES

Quality Microscopes and Accessories by BECK, of London. Also SPECTROSCOPES for Medical and Chemical purposes by the same makers.

MICROSCOPICAL STAINS

Stains by G. T. GURR, the original British Manufacturers of Microscopical Stains.

MEDIA

Media for use in Bacteriology and Mycology by "DIFCO," trade name of the pioneers, in the Research and Development of Bacto-Peptone and Dehydrated Culture Media.

SUGARS

Highest quality Bacteriological Sugars or Biochemical Reagents of the "KERFOOT" Series, manufactured by Thomas Kerfoot & Co., Ltd., of England.

CHEMICALS

A wide range of CHEMICALS, by various makers and of Analytical and Laboratory Grades, is available from Stock.

GLASSWARE

Interchangeable Glassware with Ground Joints by QUICK-FIT & QUARTZ. Minimize contamination and breakages by use of Quality All-Glass Apparatus. Items of routine interest, such as test tubes, Petri dishes, beakers and flasks available from stock.

HEATING EQUIPMENT

All types of Standard Laboratory Heating Equipment by TOWNSON & MERCER, of Australia. Consult us on that special heating problem.

TOWNSON & MERCER (N.Z.), LTD.,

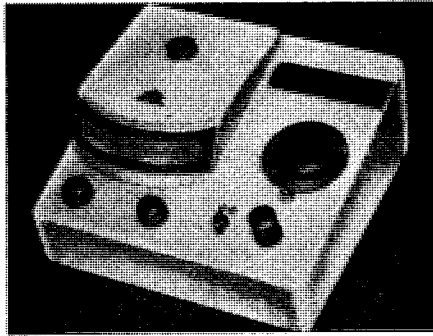
124 LICHFIELD STREET, CHRISTCHURCH

P.O. Box 1254 Phone 30-919 Telegrams: Townmer



UNICAM INSTRUMENTS (CAMBRIDGE) LTD.

Spectrophotometers, Colorimeters, Microtomes, Goniometers,
Galvanometers and other high grade instruments.



The Unicam "D.G." Spectrophotometer.

Catalogues and quotations on request from the Sole Agents
for New Zealand.

GEO. W. WILTON & CO., LTD.

Box 1980,
63 Shortland St.,
Auckland, C.1.
Phones: 41-795
44-921.

Box 367,
156 Willis St.,
Wellington, C.1.
Phones: 53-504
53-505.

JOURNAL
of the
NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

Editors: D. Whillans, Auckland,
H. T. G. Olive, Wellington, and M. O. Ekdahl, Hawera.

Vol.5. - No. 3.

July, 1950

EDITORIAL

The time has come to consider the future of this Journal. When it started almost five years ago it was hailed as a bold experiment, and it can be confidently claimed to be soundly established now.

However, your Editor-cum-printer and publisher, finds the work of producing the Journal more than he can now manage and would like to see it produced commercially. Without doubt this will mean a fair increase in the present small subscription, but in this way all can share in the production of this paper.

The Editor should be replaced by an Editorial Committee in the town in which the Journal is produced so that the work can be spread over a number of people. Your present Editor is now working in too small a field for his knowledge to progress with the times over the wide range covered by this Journal. He also has a growing family whose claims to his time should be met.

The present press should be maintained, as much supplementary material may well be printed, and it is suggested that if the January issues are eliminated, the Journal would be much easier to produce on time. This matter merits your earnest attention.

STAPHYLOCOAGULASE

(IAN D. SCOTT)

(*From the Pathology Dept., Christchurch Hospital*)

During the last decade or more an enormous aggregation of literature covering the Staphylocoagulase reaction has accumulated. Much of it is of a conflicting nature and it is the purpose of this paper to attempt to present some of the relevant data. Numerous works on the subject have been studied and an endeavour has been made to bring the most practical points to the fore. A summary of a series of observations which have been made here will also be presented.

Since the phenomenon of the coagulation of plasma by staphylococci was first observed by Loeb (1903), many theories have been put forward as to its mode of action and its significance. In order to understand the significance of the Staphylocoagulase reaction it is as well to have some knowledge as to its mode of action.

Mode of action.

The most interesting theory in this direction is that put forward by Smith and Hale (1944). These workers submit that Staphylocoagulase is the precursor of a thrombin-like substance capable of converting fibrinogen into fibrin clot in the presence of an "activator" contained in plasma. Most workers agree, in part, that this is the case. It is also held that the substance itself is inactive and requires the presence of the "activator" substance before clotting takes place.

Occurrence of Staphylocoagulase.

As the name implies, the substance is one produced by organisms of the genus *Staphylococcus*. It has been found by experience, that only pathogenic members of this genus possess the property of producing Staphylocoagulase. It is also known that the majority of these pathogens are individuals of the strain commonly termed *Staphylococcus aureus* or "albus" variants thereof. One worker (Fairbrother, 1940) found four "albus" strains to be coagulase-positive but these were probably non-pigmented strains of *Staphylococcus aureus*. None of the literature examined gave instances of *Staphylococcus citreus* producing Staphylocoagulase and no reference could be found regarding the behaviour of *Staph. cereus flavus*, *Staph. cereus albus*, or *Staph. ascoformans*, in this direction. As these last three organisms are very seldom encountered, no opportunity of testing them presented

itself.

Staphylocoagulase in relation to pathogenicity.

The vast majority of workers now regard an organism which produces Staphylocoagulase as pathogenic or potentially pathogenic. Chapman et al (1937) found that this is true in at least 98.9% of cases. In their series, several other recognised criteria of pathogenicity were used in parallel with a tube test for Staphylocoagulase. Smith, et al (1947) found a definite relationship between alpha-toxin and Staphylocoagulase in their recent research on the pathogenicity of Staphylococci and the production of Staphylocoagulase.

Methods for the demonstration of Staphylocoagulase.

Methods, almost as numerous as the articles covering the subject, have appeared at various times. Of these, the most satisfactory would appear to be that of Fisk (1940), who conducted a series of very practical tests covering every phase of his method. Although not as rapid as the slide techniques described by Cadness-Graves (1943) and Berger (1943), the tube method of Fisk is the one of choice, and is used in this laboratory.

The Method of Fisk (1940)

Minor alterations of some details have been made for our own convenience, but the principles of the test remain as in the original monograph.

Materials Required.

1. Sterile Wasserman tubes.
2. Sterile Pasteur pipettes to deliver 40 drops per ml.
3. Sterile graduated 1.0 ml. pipettes.
4. Sterile graduated 10.0 ml. pipette.
5. Kahn rack or similar rack.
6. Rubber teats.
7. Sterile plasma (Human).
8. Sterile Physiological Saline.
9. Broth culture Staph. aureus—coagulase positive.
10. Broth culture Staph. aureus—coagulase negative.

Technique

(a) Number the tubes against the cultures to be tested and include three extra tubes labelled (1) "+" = positive control, (2) "-" = negative control and (3) "B" = blank.

(b) The plasma is diluted to 1 in 10 with physiological saline and thoroughly mixed.

(c) Measure 0.5 mls. of the diluted plasma into all of the

tubes.

(d) Into the appropriate tubes place five drops of the corresponding broth culture. Both controls are treated in a similar manner. The blank does not receive organisms as it acts merely as a control for detection of spontaneous coagulation of the plasma, which occasionally occurs.

(e) Place the rack and the tubes in the water-bath at 37°C.

(f) Observe all the tubes every 30 minutes during incubation up to six hours, for evidence of coagulation of the plasma.

Reading the Results.

A positive result is recorded when the plasma has formed into a firm jelly-like clot which will remain in the bottom of the tube when the tube is inverted. Early coagulation is shown by a fine "reticulated" appearance somewhat similar to the "spider-web" clot observed in Cerebro-spinal fluid in tuberculous meningitis. Later the whole contents of the tube will be found to have completely coagulated. Positive strains usually show coagulation after 30-180 minutes, but sometimes coagulation is delayed for four-five hours. Strains which show no coagulation after six hours are regarded as negative.

Table 1 shows an arbitrary system of recording results, as used in this laboratory. The symbols represent the time in hours taken for coagulation of 10% human plasma at 37°C under standard conditions; but is not a quantitative estimation of the amount of Staphylocoagulase produced by any given strain, although it appears as though there may be a relationship between these conditions. (Smith & Hale, 1944).

Table 1.—An arbitrary system for grading results in Staphylocoagulase reactions.

Time for coagulation in hours	Symbol
0.25 — 0.5	+++
0.5 — 1.0	++±
1.0 — 1.5	++
1.5 — 2.0	+±
2.0 — 3.0	+
3.0 — 6.0	±
Over 6.0	—

Reporting.

Assuming that all strains of staphylococci which produce Staphylocoagulase are *Staphylococcus aureus* or "albus" variants of "aureus" strains, it is as well to report these organisms as "*Staphylococcus aureus* (coagulase — positive ++ etc.)", or alternatively as "*Staphylococcus pyogenes* (coagulase — positive ++ etc.)". With negative strains, the nomenclature depends

entirely on the pigmentation of the strains. Alternatively, it has been suggested (Cruikshank, 1937) that negative strains be termed *Staphylococcus saprophyticus*. This, however, is a matter of personal preference and is not critical. The most important feature is for the clinician to be enabled to determine whether the strain of *Staphylococcus* isolated, from material submitted for Bacteriological examination, is pathogenic or non-pathogenic.

The Method of Cadness-Graves (1943).

This method has the advantages of being simpler and much quicker and reliable in experienced hands, but has the serious disadvantage of requiring a considerable amount of experience in parallel with a tube method before consistent results are obtainable. An added disadvantage is that fresh plasma is necessary. False positive reactions occur with "stale" plasma. The method is useful for large scale surveys of staphylococci when used as a "screen test."

A thick suspension of the suspected colony direct from the plate is made in a drop of water on a clean slide. A loopful of fresh plasma is gently mixed with the emulsion on the slide with the loop. Coagulase positive strains of staphylococci are precipitated after 5-15 seconds, in the form of easily visible white clumps. With other strains of staphylococci the emulsion remains uniformly turbid. Cadness-Graves states that 90% of strains positive by a tube method used in parallel in her series, were also positive by the slide method. These results were obtained by experienced observers. The method is not recommended for routine use.

Further Observations.

Having detailed two methods for the demonstration of Staphylocoagulase and discussed the reaction in general, a few further details will be mentioned which have direct bearing on the performance of the test.

Plasma.

The plasma of choice is human plasma; it should be sterile, fresh and free from cells, and, if kept under sterile conditions at 0°—2°C, will keep for 6-8 weeks without noticeable deterioration. For convenience, it was found that if kept in 10.0 ml. ampoules in 1.0 ml. volumes unnecessary pipetting was avoided and the risk of contamination was reduced. Each fresh batch of plasma should be tested against known positive and negative controls.

Plasma obtained from the horse, sheep, ox, rabbit or goat is also suitable for coagulase tests. That obtained from the guinea-pig, mouse or fowl is entirely unsatisfactory (Smith & Hale, 1944), as these appear to lack the "activator" substance described

by those workers.

Pooled human plasma, prepared for transfusion, was also found to be unsuitable. This was due to the fact that such processed plasma lacks fibrinogen, thus inhibiting coagulation.

Cultures.

Cultures must be made in media free from fermentable carbohydrate, e.g., glucose. Fermenting carbohydrates present in the medium are said to promote the production of a substance termed "anti-coagulase," thus rendering positive strains negative.

It is desirable to use a young broth culture for the test (18-24 hours at 37°C). Cultures do not show any appreciable deterioration in seven days at 4°C or in three days at 15°C. Fisk (1940) has shown that cultures, in some cases, retain their power of coagulation for plasma, for some three-twelve weeks at room temperature. Other strains are stated to lose this power in seven-fourteen days.

Broth culture filtrates are said to retain their power of coagulation of plasma (Lominski & Roberts, 1946), although some workers claim that this is not the case. (Christie and Keogh, 1940). "Gradocol" membranes having an A.P.D. of 0.11 μ . are stated to retain the active principle.

Results of a Series of Staphylocoagulase Reactions on 98 Strains.

During the past few weeks a record has been kept of the strains of staphylococci tested for Staphylocoagulase in this laboratory.

The individual strains have not been enumerated but have been classified into groups according to their origin. Five groups have been collected in all and consist of those shown in Table II.

Table 2, showing number of strains isolated from various sources, classified in groups.

<i>Group</i>	<i>Isolated from</i>	<i>Aureus</i>	<i>Albus</i>	<i>Total</i>
A	Abscesses, furuncles, burns	51	3	54
B	Ear, eye, nose and throat	16	1	17
C	Bone and joint, serous cavities	13	3	16
D	Genito-urinary system	21	4	25
E	Miscellaneous	13	1	14
<i>Total</i>		114	12	126

The strains included in group E were isolated from a wide variety of lesions and include strains from blood-cultures, the alimentary system, skin and one strain from a tropical ulcer.

Table III. shows the results of the reactions given by organisms of each group. The results are expressed as percentages and as absolute figures. The series was not a very large one, but gives some idea of the number of coagulase—positive strains which may be encountered in the routine laboratory.

Table 3, showing numbers and percentages of strains coagulase positive and coagulase negative in a series of 98 strains.

Group			No.		Total
	No. Positive	Per Cent	Negative	Per Cent	
A	47	87.1	7	12.9	54
B	13	76.5	4	23.5	17
C	11	68.8	5	31.2	16
D	12	48.0	13	52	25
E	7	50.0	7	50.0	14
<i>Total</i>	90	71.5	36	28.5	126

Summary.

A review of literature covering the phenomenon of Staphylocoagulase over the past decade has been made.

Methods have been described for the performance of the coagulase test.

Various aspects of the reaction have been discussed and it has been shown that the test is a useful criterion of pathogenicity of staphylococci.

A survey of a small series of strains tested in this laboratory has been included.

References.

- | | | |
|---|----|-----|
| Berger (1943), <i>J. Path. Bact.</i> | 55 | 435 |
| Cadness-Graves (1943), <i>Lancet</i> | 24 | 736 |
| Chapman, et al (1938), <i>J. Bact.</i> | 35 | 311 |
| Idem (1937), <i>J. Bact.</i> | 33 | 533 |
| Christie & Keogh (1940), <i>J. Path. Bact.</i> | 51 | 189 |
| Cruikshank (1937), <i>J. Path. Bact.</i> | 45 | 295 |
| Fairbrother (1940), <i>J. Path. Bact.</i> | 50 | 83 |
| Fisk (1940), <i>Br. J. Exp. Path.</i> | 21 | 311 |
| Lominski & Roberts (1946), <i>J. Path. Bact.</i> | 58 | 187 |
| Smith & Hale (1944), <i>Br. J. Exp. Path.</i> | 25 | 101 |
| Idem (1945), <i>Br. J. Exp. Path.</i> | 26 | 209 |
| Idem (1947), <i>Br. J. Exp. Path.</i> | 28 | 57 |
| Topley & Wilson: Principles of Bacteriology and Immunity. | | |
| 3rd edition (1946), p. 618. | | |

PLASMA VISCOSITY.*A. FISCHMAN (Auckland)*

Blood viscosity studies were made many decades ago, but only work done in the last few years seems to suggest that they might provide a valuable practical laboratory procedure equal or perhaps even superior to one of our most important routine tests, the sedimentation rate of red cells. Originally interest was focussed mainly on whole blood viscosity (BV.), while lately more interest has been shown in determination of plasma viscosity (PV.).

Whole Blood Viscosity.

Theoretically the viscosity of a solution is a linear function of the relative volume occupied by the solute in the solution. Two constituents of blood occupy a large relative volume, the red cells and the proteins. In whole blood the relative volume of red cells is much larger than any other constituents, including the proteins. Therefore BV. will be mainly determined by the red cell volume. There is close parallelism between BV. and RC. and thus BV. has a similar significance as haematocrit values. Formulas have been devised to calculate BV. from haematocrit results and good agreement with the experimental value may be obtained. Determination of BV. is of little practical value as better methods are available for the diagnosis of anaemia and polycythaemia. Protein changes have only slight effect on BV.

Plasma viscosity.

In the absence of red cells viscosity is mainly determined by the proteins. Increase in both fibrinogen and globulin is reflected in PV. values. Albumin has only slight effect. The Chinese authors, Wang and Tang (1941), pointed out first the great value of PV. measurements in assessing clinical activity of tuberculosis. They claimed it to be superior to E.S.R. This view was confirmed by Miller and Whittington (1942) and Houston and co-workers (1945). Similarly P.V. was found to be of great value in following up rheumatoid arthritis, by Cowan and Harkness (1947), and other authors in 1948 and 1949.

Technique.

Several types of viscometers are in use, all based on the same principle. The liquid is allowed to flow through a capillary tube and the time of outflow between two marks measured. The same procedure is applied to distilled water and the ratio of flow-times used to indicate relative viscosity (R.V.). Strictly speaking, this is not relative viscosity, only an arbitrary figure. In calculating

R.V. densities also have to be taken into consideration. For practical purposes this is usually omitted. The Poiseuille-Ostwald meter is the standard apparatus in physical chemistry, it needs however, a considerable amount of blood, and therefore not very practical. Houston and co-workers (1945) modified it in a such a way that less than one ml. is sufficient for one determination. Most medical laboratories used the Hess meter in the past. This consists of two parallel capillary tubes, which are filled with blood and water respectively. Suction is applied to both tubes simultaneously and the volumes of liquids passing through the tubes during a certain time are compared. Several authors prefer a single straight capillary tube, the lower end of which is immersed in the liquid. It is filled by suction and the liquid allowed to flow back between the marks.

In our laboratory a viscometer has been used applying the principle of Denning and Watson (Whitby and Britton, 1947). It consists of a single U-shaped capillary tube. The top end of one arm is widened, the second arm has a widening on its lower part between two marks. The meter is secured vertically in a transparent water bath, which is also provided with a thermometer, electric heater and stirrer. Most authors adjust the temperature to 20 C. Thermostatic control is useful and time-saving, but not essential.

Heller and Paul's oxalate mixture is used for obtaining whole blood and plasma. 1.5ml. is needed for a determination in our meter. The blood or plasma is kept at the temperature of the water bath for about four minutes, and then introduced into the top of the widened arm. The time required for the liquid to pass between the marks is accurately measured with a stopwatch. The flow-time of water is determined in a similar way. The average of three measurements is calculated. The flow-time of water for a certain temperature has to be determined on one occasion only. With accurate work flow-times of the same sample at a certain temperature should not vary more than 0.1 to 0.4 per cent. If the error is greater, faulty technique should be suspected. Possible causes of error are wrong timing, temperature control, or insufficient care in cleaning the instrument. The smallest particle adhering to the inner wall of the tube might increase the error. After every measurement cleaning with water, alcohol and ether followed by current of air is essential. Occasionally chromic acid solution or HCl-pepsin mixture should be left in the meter which is kept in the incubator overnight.

Colloid calibration.

As most workers use different meters, some difficulty arises when comparing results. Pure liquids have identical viscosities in any meter. Colloidal solutions, however, are anomalous and do not give exactly the same figures in different instruments. Houston

and co-workers (1945) examined this problem in great detail. They thought that either all instruments should be made as closely as practicable to a standard size, or a specially selected colloid solution be used for comparison instead of water.

It is recommended in any case to have several meters in the laboratory, and viscosities of several samples of plasma determined with all instruments. In this way the difference between these is obtained, and if the one in constant use breaks, it is possible to express results in terms of the original apparatus. If that is not done, even one's own results are not comparable.

Results.

The calculation of absolute units is somewhat laborious and is seldom done for practical purposes. To calculate relative units RV. of water is considered 1.00 or 100. In the course of our three years' study of the subject the normal range of PV. was found to be 1.56 to 1.81 (or 156 to 181). This coincides with results of some authors, but shows disagreement with others. The difference can be only partly due to the use of various types of instruments. It is important that agreement be reached on the upper limit of normal as the practical application of PV. depends on that to a great extent (Fischmann and co-workers, 1950).

PV. is raised in more than 90 per cent of cases with active rheumatoid arthritis. Thus PV. shows a similar behaviour to ESR. Values range from 180 to 240. Variation in PV. is in direct proportion to the degree of clinical activity. Some workers believe that in many cases PV. is a better indicator of clinical status than ESR.

Latest developments in this field are: 1. Application of PV. in other pathological conditions where protein abnormalities may be present. 2. Measuring viscosity of different fractions of plasma. While this work is still in the initial stages, it promises to be of even greater practical value than simple plasma viscosity.

Summary.

Determination of plasma viscosity is a new clinical laboratory method of considerable practical value. Technique of measurements and range of normal and abnormal values are discussed.

Acknowledgment.

Thanks are due to Dr. E. J. Fischmann, physician, Auckland, and Mr. F. H. Sagar, M.Sc., Auckland University College, for permission to publish this article.

References.

- Cowan, I. C., and Harkness, J. (1947), *Brit. Med. J.*, 2, 686.
Fischmann, E. J., Sagar, F. H., and Fischman, A. (1950), to be published.

- Houston, J., Harkness, J., and Whittington, R. B. (1945), *Acta Tuberc. Scand.* 19, 153.
Miller, A. K., and Whittington, R. B. (1942), *Lancet*, 2, 510.
Tang, H. V., and Wang, H. (1941), *J. Trop. Med. Hyg.*, 1, 28.
Whithy, L. E. H., and Britton C. J. G. (1947), *Disorders of the Blood.*

A MODIFIED SOMOGYI DIASTASE METHOD.

(F. M. HILDER)

(From the Pathology Dept., Christchurch Hospital)

In most N.Z. laboratories the Wohlgemuth method is used for serum and urine diastase estimation. The usual form of this method suffers from one serious disadvantage. Owing to the use of a doubling dilution technique it fails to detect slight increases in the diastase level—for instance between 32 and 64 units. More complicated dilution methods may be employed, but they are tedious and time consuming.

It is thus quite satisfactory in cases of acute pancreatitis, but is difficult to apply to the diagnosis of chronic pancreatitis where the level may be but slightly increased.

The introduction of a prostigmine stimulation test (1) for chronic pancreatitis which involves five serum diastase estimations and slight elevations of the level if positive, led us to investigate other methods.

We found that Somogyi uses both a copper reduction and an iodine and starch method (2). The simplicity of the latter method commended it to us. Somogyi employs a unit based on the amount of reducing sugar produced by the enzyme and standardizes his iodine and starch against his copper reduction method. The normal range in Somogyi units is 80-150.

To avoid the complication of introducing a new unit we standardized a modified Somogyi iodine and starch method against the Wohlgemuth method. Normal range 4-32 units.

Although appearing on first acquaintance to be more complicated, we have found this method to be simpler in practice, more accurate and a great time saver when a number of estimations have to be performed at one time.

Reagents.

0.15% Starch Solution.

Weigh accurately 150 mgm soluble starch (Analar or other good quality) and transfer to a small flask. Add 90 mls. dist.

water and just bring to boil.

Cool and make up to 100 mls. volumetrically. Keep in refrigerator.

0.5% Sod. Chloride Solution.

0.002N Iodine Solution.

Dilute 10 mls. of 0.1N aqueous iodine soln. to 500 mls. with 2% Pot. iodide soln. The 0.1N iodine need be approximate only and may be prepared by dissolving 25 grams pot. iodide in 200 mls. water, adding 12.7 grams iodine to this, shaking till solution is complete and finally making up to 1 litre with dist. water.

Method.

Pipette into test tube 2 mls. 0.15% starch soln. and 2 mls. 0.5% sod. chloride soln. Mix and place in water bath at 37°C.

Set up 5-10 small Kahn tubes in a rack and pipette into each 0.5 mls. 0.002N iodine.

When tube containing starch soln. has been in bath about five minutes add 1 ml. serum (or urine) rinsing pipette out with soln. and mixing. The pH of urine to be tested should be between 6.1 and 6.5. Take time accurately, preferably with stop watch.

After 1 min. has elapsed withdraw 0.5 mls. and add it to one of the small tubes containing 0.5 mls. iodine.

If the diastase index is less than 100 units a blue colour indicating the presence of undigested starch will appear. 0.5 ml. samples should then be withdrawn at two or three or five or 10 minute intervals and added to tube of iodine. A little experience soon enables one to judge from the depth of the blue colour the amount of starch still present, and to decide at what intervals of time 0.5 ml. samples should be withdrawn.

When the digestion of starch is nearing completion the red-brown of erythrodextrin will begin to appear, mixed with the blue tinge of starch. When all blue colour disappears the end point has been reached and the time should be accurately noted.

The index is arrived at by dividing the time taken to digest the starch into 100. $100/\text{Time} = \text{Diastase index}$ in Wohlgemuth units. It will be seen that if the end point has not been reached in 10 minutes the diastase index will be under 10 units, and unless great accuracy is required, withdrawals of samples at five-minute intervals will then be all that is necessary.

If the diastase index is greater than 100 units no blue colour will be produced by the 0.5 ml. sample withdrawn after 1-minute incubation.

A warning here however. Observe the iodine soln. immediately the sample is added. Sometimes a transitory blue colour will appear but fade quickly. In such cases starch is not absent.

but has been decolourised by substances in the serum or urine and a permanent blue colour can be developed by adding more iodine.

If when more iodine is added no blue colour develops the index is over 100 units and a 1 in 10 or a 1 in 20 dilution of the serum or urine must be made and a new test set up using the dilution.

Summary.

A modified Somogyi method of diastase estimation is described which has been found in practice to have many advantages as a standard technique.

Acknowledgments.

This work has been carried out in the Biochemical laboratory of the Christchurch Hospital Pathology Department, and thanks are due to Dr. D. T. Stewart, Director for permission to publish this article, and to Mr. J. T. Murray, Biochemist for his help and encouragement.

References.

- (1) Knight, Muethers and Sommer. *Gastroenterology*, Vol. 12. No. 1, page 34.
- (2) Somogyi M. *J. Biol. Chem.* Vol. 125. Sept., 1938, page 399.

DEPARTMENT OF HEALTH, NEW ZEALAND. INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES.

**Pathology Department, Christchurch Hospital, Friday
and Saturday, 26th and 27th May, 1950.**

Paper.

Friday, 26th May, 1950—9.30 a.m.

Time allowed: Three hours.

- (1) How would you prepare a normal solution of Caustic Soda (N. NaOH)? (15)
- (2) Give two common examples of the Neisseria group. In what specimens would you expect to find them and how would you proceed to identify them? (20)
- (3) Describe one method for determining the T.N. P.N. of a sample of blood and explain the chemical princi-

ples involved. (15)

(4) Describe in detail the performance of a red cell count on a sample of oxalated blood.

Discuss the sources of error involved in such an estimation and compare the accuracy with that of determination of packed cell volume by the haematocrit. (20)

(5) What means of sterilisation are available in a hospital laboratory? Briefly describe the apparatus appropriate to each method you mention, indicating the underlying principle and one routine use in each case. (20)

(6) Write brief notes on:—

- (1) Neutropenia.
- (2) Xanthochromia.
- (3) Detection of occult blood in faeces.
- (4) Detection of albumin in urine. (10)

Practical.

FRIDAY, 2.30-5.30 p.m.; SATURDAY, 9 a.m. onwards.

Time allowed: 35 minutes in each group. A maximum of 25 points to be allotted for each group.

Instructions: Candidates will be divided into four groups. A signal will be given at the end of 35 minutes, when groups will change over, five minutes being allowed. At the end of the second change there will be a break of 20 minutes. Saturday morning from 9 a.m. will be allotted for final work on cultural examinations, etc., in Group 2a.

Label all practical work with your examination number.

Where instructed leave all material prepared on card and put your number on right hand top corner.

GROUP I. (a) Identify organisms growing on blood agar plates. No sub-culturing required. These are 24-hr. cultures.

(a) *S. aureus*, *Str. haemolyticus*, *N. catarrhalis*, *Ps. pyocyanea*.

(b) Write brief notes on slides, labelled 1, 2, 3, 4.

(b) *Vincent's organisms*, *streptococci in C.S.F.* *Gram-ve bacilli in urine*, *Myco. tuberculosis in sputum*.

GROUP II. (a) Identify organism growing on plate of MacConkey medium.

(a) *S. paratyphi A*.

(b) From the materials provided prepare:

- (i) a blood agar plate and slope.
- (ii) three throat swabs in plugged tubes.

(b) 20 mls. melted agar at 48°C and bottle of blood, and 1 ml. pipette.

GROUP III. (a) Carry out microscopical examination and tests for albumin and sugar on urine supplied. (Stained film not required).

Casts, leucocytes and yeasts. Albumin and sugar positive.

(b) Estimate free HCl and total acid in specimen provided, expressing your results as the No. of cc. N/10 acid present in 100 cc. of sample.

Free HCl; 38 cc. Total acid 60 cc.

GROUP IV. (a) Type specimen of citrated blood supplied.

Group B.

(b) Carry out leucocyte count on blood supplied.

(c) Prepare two films from blood IVb.

(d) Recognise the abnormality of leucocytes in film X. (No differential count required).

Lymphatic leukaemia.

(e) Describe *briefly* red cell picture in film Y.

Microspherocytes and polychromatic macrocytes (in an acholuric jaundic).

HERE AND THERE

Our congratulations go to Dr. A. B. Pearson, C.B.E., Pathologist, of Christchurch, on his recent honour. Dr. Pearson has seen the whole of Pathology and its allied sciences grow to their present state in New Zealand, and after many years of service in the Pathology Department of the Christchurch Hospital, is now in private practice with his son, Dr. C. T. B. Pearson.

Mrs. L. Isabeth, formerly at Gisborne, has taken charge of a new Laboratory at Wairoa Hospital. Mrs. Isabeth commenced work in the Cook Hospital Laboratory in 1936, and except for the period 1943-1945, when she was training in the Auckland Hospital Laboratory, has served there continuously. Wairoa Hospital is 66 miles south of Gisborne.

In Mrs. Isabeth's place is Miss Helen Schrender, M.Sc., of Melbourne University. She gained her M.Sc. in 1945 with a thesis on the Bacteriological Diagnosis of Typhoid Fever, and since graduation has worked in several hospital laboratories in Australia.

Mr. W. Carruthers, whose name is almost synonymous with that of the Cook Hospital Laboratory, is leaving there after 30 years' service, to

be Bacteriologist at the Queen Elizabeth Hospital for Rheumatic Disorders, Rotorua. He will commence work there on August 7th, 1950. Our best wishes go out to him in his new venture.

Entries for the Junior Essay and Technical Essay Competition close with the Secretary on Friday, July 28th, 1950. A good entry is desired. There is no entrance fee. For further details see the Rules and their amendments.

The Director-General of Health draws attention to the position of Relieving Laboratory Superintendent at Suva. Mr. Perry Johnson will go on furlough for about twelve months in November next and qualified Hospital Bacteriologists are invited to apply. Salary is up to £720 p.a. (Fiji), and further details may be obtained from the Health Department.

In a recent reserved judgment, Mr. Justice Hutchinson found: "In my opinion the operation of hospitals is not a related industry to the operation of hotels, restaurants and the like." It is hoped to cover the whole of the conditions leading up to the Court action at the Conference, and a full report will appear in the next Journal. This judgment dealt with the question whether hospital technicians and male nurses could properly be included in the Hotel, Hospital, Restaurant and Related Trades' Unions, and his Honor found that the word "Hospitals" should not have been added to the title of the union. As it was felt that the freedom of action of Hospital Bacteriologists and Laboratory Assistants was in question the New Zealand Association of Bacteriologists, through its Council has pursued a vigorous course with the above very desirable result.

A few complete copies of this JOURNAL from Vol. 1, No. 1, are still available and may be obtained from the Editor. The prices are as usual at the rate of 5/- per year.

AN IMPORTANT ANNOUNCEMENT

**TO ALL PATHOLOGISTS, BACTERIOLOGISTS,
BIO-CHEMISTS AND HOSPITAL LABORATORY
TECHNICIANS**

W. LITTLEJOHN & SON, LTD.,

OF 224 LAMBTON QUAY, WELLINGTON, C.I.
(Telephone 40-249. P.O. Box 186. Telegrams "Littlejohns")

DESIRE TO ANNOUNCE—

their entry into the Science Laboratory Apparatus and Chemical Supply Trade. Suitable shop premises and a good range of stocks will be available at an early date. Overseas Indent Orders for all Hospital Laboratory needs can now be accepted, on a most attractive basis, worthy of your immediate attention.

The entire Science Department is under the management of Mr. A. J. H. McClay, late Sales Representative of Messrs. G. W. Wilton & Co., Ltd., Wellington.

We will welcome enquiries and personal calls and if you wish to be on our regular mailing list, will you kindly forward us your name and address?

The brand new (6th) edition of this important book is scheduled to leave the English press in June, 1950. Please order your copy now. Tentative price, 52/6.

DISORDERS OF THE BLOOD: Diagnosis; Pathology; Treatment and Technique.

By Sir Lionel Whitby, C.V.O., M.D., F.R.C.P., Regius Professor of Physic, University of Cambridge, and C. J. C. Britton, M.D., D.P.H., Consulting Haematologist, Prince of Wales' General Hospital, London.

6th Edition. 750 pp. Roy. 8vo. 12 coloured plates and 94 text-figures.

The preparation of a new edition of this standard text-book of haematology is a formidable task in view of ever-changing opinions and rapid advances in knowledge in such subjects as coagulation, corpuscular antigens, and specific therapy. In these aspects of the subject, and many others, there is now an almost boundless literature to survey, necessitating a most careful selection for the bibliography.

This edition has called for an extensive revision of the whole book, but particularly those chapters dealing with haemopoietic metabolism, coagulation, the haemolytic anaemias, leukaemia, haemagglutination, pigment metabolism, and on Technique. The type has been entirely reset and the text shows a considerable increase in extent. Four new coloured plates have been included, mainly derived from photomicrographs in colour, and 23 other illustrations have been added.

Reviews of the Fifth Edition said:—

"It is the outstanding British work on haematology." — *British Medical Bulletin*.

"This outstanding work has become the recognised standard in the subject."—*Edinburgh Med. Journal*.

"This volume holds pride of place as the best British text-book on haematology."—*Medical Journal of Australia*.

ON SALE BY—

N. M. PERYER, LIMITED,
Medical Publishers, 145-147 Worcester Street,
CHRISTCHURCH, C.1.



*Pharmaceuticals
and Fine Chemicals*





Suppliers of

MICROSCOPES and ACCESSORIES

BECKS—London.

SPENCER LENS, Binoculars.

KLETT Colorimeters, Spare Cups and Plungers.

HELLIGE Haemometers.

SPENCER Bright Line Haemocytometers.

Haemocytometer Cover Glasses.

Haemocytometer Pipettes, Red and White.

SAHLI Pipettes for Haemometers.

Microscope Slides, 3 x 1. Plain and with cavities.

Microscope Cover Glasses.

Lens, Paper, Lamps, Stains, Slide Boxes.

Mounting Media.

Culture Loops with platinum wire.

Dissecting Needles. Slide Labels.

Seekers, Chinagraph Pencils.

**LUER Tuberculin Syringes and Hypodermic Syringes and
Needles, all sizes.**

Scalpels, Dissecting Forceps.

An Excellent Assortment of Bacteriological Reference Books.

Laboratory Glassware. Scientific Apparatus.

HOPKIN & WILLIAMS Analytical Reagents.

KEMPTHORNE, PROSSER & CO.'s

New Zealand Drug Company Limited.

22-26 STAFFORD STREET, DUNEDIN.

Auckland, Wellington, Christchurch and Dunedin.