Vol. 14, No. 1 APRIL, 1959

JOURNAL

OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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Vol. 14, No. 1 April, 1959

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All moneys should be paid to the Treasurer of the New Zealand Association of Bacteriologists (Inc.), c/o. Mr M. McL. Donnell, 17 Anzac Street, Takapuna, Auckland N.

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ADDRESSES

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THE IMPORTANCE OF ENZYMES IN HOSPITAL LABORATORY PRACTICE

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The importance of enzymes in hospital laboratory practice is greater than is generally realised, and with the rapid advances now being made in this field it will probably assume a greater significance in the future. Fermentation typing of micro-organisms, clotting tests, certain methods of urea estimation and tests for pancreatitis are all in the province of enzymology. However, the action of these remarkable compounds is seldom considered, and it may be even reasonable to say that we are often unaware of 'what is going on'.

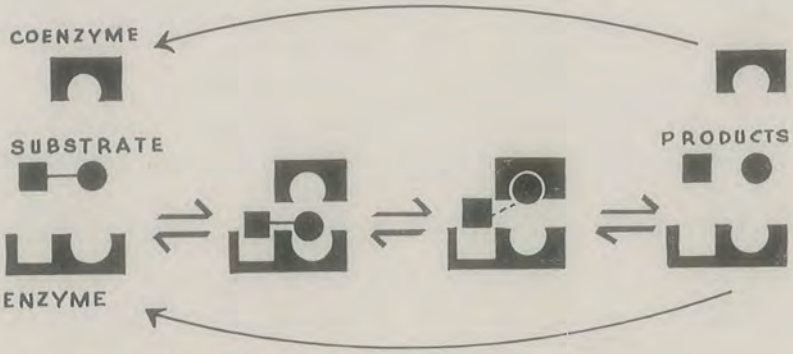
Certain features of enzymology have already been dealt with in this Journal, McLean (1), Kennedy (2), King (3); whilst the enzymes of the blood in general have been well reviewed by Bruns (4).

THE NATURE AND MODE OF ACTION OF ENZYMES

Enzymes are mostly complex proteins, as a result they are unstable when subjected to extremes of temperature and pH. Furthermore, they have optimal physical conditions for their functioning and often require the presence of cofactors (coenzymes) and metal ions.

The enzymes catalyse chemical changes within the body, and like the inorganic catalysts they do not increase the yield of products but rather hasten the rate of reaction. In fact the enzymes may work 'backwards' under some conditions to form the forerunner substance again from the products if the equilibrium favours that direction of reaction.

The outstanding thing about the enzyme, however, is its marked specificity. Whereas a given inorganic catalyst, say, platinum, will catalyse a host of reactions, the enzyme will catalyse only one, or in some cases a very limited range of closely related reactions. It is thought that the enzyme and substrate combine by means of their spatial arrangements of side chains and groups—hence the specificity. Then a 'loosening' of the substrate components occurs, the coenzyme then accepts the products and frees the enzyme to attack more substrate. The products are then given up by the coenzyme. Many of the enzymes have their coenzymes firmly bound to them—for example the glutamic-oxalacetic transaminase, Shepherd and McDonald (5). A schematic reaction using enzyme and coenzyme is shown in Fig. 1.



TYPES OF REACTIONS

A few examples of enzyme catalysed reactions follow, to which reference will be made again later.

1. *Hydrolysis*

When foodstuffs are digested, or carbohydrate stores in the body are broken down, it is by a process of hydrolysis. Thus, the salivary enzyme ptyalin will form a disaccharide—maltose—from the polysaccharide starch. Then, later, the pancreatic enzyme maltase will split the maltose into glucose units which are readily absorbed.

(i) Starch + H_2O $\xrightarrow[\text{amylase}]{}$ Maltose + Glucose

(ii) Maltose + H_2O $\xrightarrow[\text{maltase}]{}$ Glucose + Glucose

This type of enzyme is called a "glycosidase".

2. *Phosphatases*

There are a number of phosphatases, some having very specific substrates, others being able to split off phosphate from a range of substrates. The reaction given here is one important in intermediary metabolism. Phosphatases are important in connection with the absorption of monosaccharides from the intestine, reabsorption of glucose in the kidney, and for the formation of bones and teeth.

(iii) Glucose-6-Phosphate $\xrightarrow[\text{phosphatase}]{}$ Glucose + phosphate

3. *Transaminases*

Another general type of reaction is the 'group transfer' described here with particular reference to glutamic-oxalacetic transaminase. In this reaction, a complete amino group is removed from one substrate, and passed to another. This reaction is of great importance, since it is the means whereby a protein in the body is broken down into a carbohydrate skeleton for fuel, and

the nitrogen is passed on to be formed into urea. In this way nitrogen is removed from the tissues and formed into urea without the formation of toxic quantities of ammonia.

(iv) α— amino acid + α— ketoglutarate $\rightleftharpoons$ (transaminase)

glutamate + α-keto acid.

Here the α-ketoglutarate is the acceptor of the amino group.

Apart from the obvious importance in intermediary metabolism, there is a laboratory application of this reaction. (See later.)

LABORATORY TESTS

Having briefly reviewed the nature, and a few reactions of enzyme systems, we may now consider some of the laboratory tests in which enzyme activity is estimated.

1. *Serum amylase reaction*

As previously described, amylase can split starch to give maltose. In the test, serum is serially diluted and added to tubes containing starch solution. The tubes are maintained at the optimal temperature for the enzyme for a standard time. For enzymes in human material the optimal is in the range 37°C. Next, iodine is added, and the presence or absence of starch (as indicated by the iodine) gives an index of the activity of the enzyme. A raised level indicates obstruction of the pancreatic ducts. Harrison (6) describes methods for the estimation of serum and urinary amylase.

2. *Serum phosphatase reaction*

Acid and alkaline phosphatases are commonly estimated, and give a good example of the necessity for using optimal conditions, for at the optimal pH of one, the other is quite inactive. Thus for acid phosphatase the pH range is 5.0-5.5, and for alkaline phosphatase 8.6-9.4.

In either case, the serum is incubated with a buffered substrate of the correct pH, and controls are also run. The substrate may be a glycerophosphate, in which case the liberated phosphate is measured; or one may use a phenyl phosphate and estimate the phenol liberated.

Acid phosphatase: only a small proportion of the serum acid phosphatase is of prostatic origin, and the literature on this subject has been reviewed by Bodansky and Bodansky (7), however, a method devised by Fishman et al (8) (9) based on the inhibitory action of l-tartrate on the enzyme of prostatic origin, has been shown useful in the diagnosis of prostatic cancer in

patients with normal values of 'total' serum acid phosphatase. This method, since it unmasks an otherwise unnoticed high level of the prostatic enzyme may well be a useful test in early diagnosis.

Alkaline phosphatase: the estimation and significance of this test are well dealt with in the standard texts, but an interesting adjunct to this determination on patients with hepatic tumours is described by Shay (10). He couples the test with the bromsulphalein test and shows that it is especially useful in indicating the operability in such cases.

3. *Serum transaminase reaction*

There is in normal human serum a low level of transaminase activity. The two enzymes responsible are glutamic-oxalacetic transaminase (GO) and glutamic-pyruvic transaminase (G.P.). Cohen (11) showed that the concentration of the enzymes were greatest in the heart, and in decreasing abundance as follows: skeletal muscle, brain, liver, kidney, testis, lung and spleen.

It was also shown by Karmen et al (12) (13) that the serum transaminase activity rises with regularity within 12-24 hours in acute human myocardial infarction, and returns to normal within 3-6 days. The level may be raised from 2-20 times that of the normal, however, high levels may also occur in cases of jaundice with active liver disease. Thus it would appear that this estimation could be a valuable test in the diagnosis of myocardial infarction. A survey carried out by Chinsky et al (14) in which the estimation was carried out on cases of the major disease categories is summed up as follows:—

'The transaminase activity is raised particularly in coronary and hepatic states, almost all the patients with acute myocardial infarction developed a significantly raised transaminase activity.' He goes on to say 'at the present state of knowledge the serum transaminase assay, when carried out at the appropriate time is most useful in excluding the presence of significant myocardial infarction or hepatic necrosis.'

As may be seen from the reference dates, this is a recent development, and we may expect to have increasing demands for this test from practitioners and housemen in the near future.

What then are the methods available for making this estimation? Unfortunately, there is at present little agreement as to the suitability of the various methods and few are simple.

The two enzymes cited here are respectively concerned with the transfer of α-amino groups of aspartic acid and alanine to α-ketoglutaric acid resulting in the formation of oxalacetic and pyruvic acids, glutamate being the second product in both cases.

(v) α-ketoglutarate + aspartate $\rightleftharpoons$ glutamate + oxalacetate.
G.O.

(vi) α-ketoglutarate + alanine $\rightleftharpoons$ glutamate + pyruvate.
G.P.

The method is to incubate serum with the substrates shown on the left in (v) and (vi). Then the oxalacetate and pyruvate are estimated. In a method described by King (15) the products are treated with 2, 4, dinitro-phenyl-hydrazine, which forms coloured hydrazones which can be measured by spectrophotometry. However, oxalacetate is not stable in presence of metals, moisture, and heat, so that it partly decomposes to pyruvate. The colour intensities of the two hydrazones are different and hence may give rise to variable results. Methods have been devised, Cabaud et al (16) (17) where the oxalacetate is converted entirely to pyruvate, the hydrazone of the latter is then extracted in toluene to separate it from the α-ketoglutarate. The optical density of the hyrazone is then measured.

King (14), in his method seeks to simplify the technique and overcome fading (which occurs in Cabaud's method) by putting up a 'blank' containing oxalacetate and pyruvate, and allows oxalacetate and pyruvate to form in the 'test' sample. He then assumes that the decomposition of oxalacetate in the 'blank' and 'test' samples is comparable. However, since there are metal ions (and other catalysts) in the serum, it is possible that the two rates of decomposition are not comparable. Indeed, King noted variable results when using plasma from citrated, oxalated, and heparinised bloods. This is probably due to the ions present, since Karmen et al (12) obtained the same values for plasma from different anticoagulant sources. This, however, might be overcome if inactivated serum were placed in the 'blank' tube at the outset. The method of Karmen (12), however, involves paper chromatography, and U.V. spectrophotometry, it is not regarded as being a suitable method for routine work, although it is very accurate. Most interest centres around the methods of Cabaud.

The enzyme activity was shown to be greatest in the α_2 globulin fraction, Shepherd and McDonald (5). Estimation of activity in urine is of no avail since there is little or none excreted by the kidney, the main pathway for the (rapid) excretion being the bile duct, Dunn et al (18).

As far as the author is aware the test is not widely used in this country, although preliminary experiments are under way.

PUTTING ENZYMES TO WORK

When we add urease to a blood sample to estimate the urea present by splitting off CO_2 and ammonia we are putting an enzyme to work, so also are we when a staphylococcus is put to coagulate serum. The enzymes used in clotting mechanisms are familiar to us all. When one considers that the numbers of enzymes in the body are legion, and that a host of disorders of metabolism are mediated through them, one cannot help but become 'enzyme conscious'. The very fermentation reactions with which we type micro-organisms are due to the enzymes which the strain typically produces and is it not annoying when a strain produces a new enzyme and gives atypical reactions? Surely we must be impressed with the facility therein to synthesise new ferments, but there we are in the realm of enzyme induction and protein synthesis.

SUMMARY

A brief survey of enzyme reactions in the laboratory has been made, and the mechanism of their action outlined. The general principles underlying some of the better known tests have been dealt with, and a review has been made of a more recent enzyme assay.

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A MICRO METHOD OF ESTIMATING PROTHROMBIN IN CAPILLARY BLOOD

G. MOYLE

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The withdrawal of venous blood for the estimation of prothrombin using the Quick method may be extremely difficult to obtain in infants and some adults especially when three or more prothrombin estimations a week are required on infants.

The micro method I have used, using the Innes and Davison technique, gives results very similar to that of Quick and has allowed results to be obtained at the bedside and also on difficult patients.

METHOD

A standard graduated white cell pipette is used for measuring all solutions. A separate pipette is used for each prothrombin estimation unless the pipette is thoroughly cleaned after each estimation.

A solution of 1.34% sodium oxalate is drawn up to the first mark on a white cell pipette. With the oxalate in position in the pipette, blood from finger prick is drawn to the 10th mark, i.e., 1 part of sodium oxalate and 9 parts of whole blood. Expel this mixture onto a microscope slide or watch glass and mix with a fine glass rod.

Draw up in the same pipette thromboplastin solution to the 10th mark and expel into the blood oxalate mixture. Mix as before.

Again using the same pipette draw 0.27% calcium chloride to the 10th mark. Start a stopwatch as soon as the calcium chloride is expelled into the blood thromboplastin mixture.

The time interval from the addition of the calcium chloride to the formation of the clot gives the clotting time in seconds.

I have found the clot is most easily seen by placing the watch glass on a white surface and slowly stirring the mixture with a fine glass rod until the clot adheres to the glass rod. The end point is quite sharp and with little practice duplication of results is easily obtained.

As a control I use my own finger prick blood, which has the advantage of using the same control each time and easily detects any abnormality of the reagents or technique.

I have found warming reagents to 37°C does not influence the results and adds a difficulty when carrying out the estimation

at the bedside. By comparing patients and controls prothrombin clotting time at room temperature within reasonable time of each other temperature variations will not influence the results. If estimations are required over a period of days I find giving the Prothrombin Index less confusing than the actual prothrombin time figures.

The Prothrombin Index being calculated by

$$\frac{\text{Prothrombin clotting time of normal blood}}{\text{Prothrombin clotting time of patient's blood}} \times 100 = \%$$

With a little practice good accuracy can be obtained. The use of small quantities of whole blood will not give as accurate results as the Quick technique but with practice very similar results will be obtained and with infants may be the only method that can be used.

SUMMARY

A micro method of estimating prothrombin in whole blood using a finger prick technique and the method of Innes and Davison is described.

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A CASE OF CIRCULATING ANTICOAGULANTS

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Patient: 71 year-old-old male.

History: Prior to his first admission to hospital on October 13th, 1955, the patient had spontaneous bruising on his limbs and back for the previous two months, followed by anorexia and increasing pallor. There was chest pain and collapse on the day before admission, when pallor became marked. He had a melaena shortly after admission.

There was no history of spontaneous bruising nor of excessive bleeding prior to the above. There was no history of a bleeding disease in his family. The patient had had a large leg wound as a youth with no bleeding trouble.

On examination he was pale and shocked. There were large bruises on his left thigh.

Investigations: Haemoglobin on admission 3.7 g/100 ml. Total and differential white counts normal. Platelet count 274,000, reticulocytes 13%. Platelet morphology—normal.

Clotting Mechanism: Bleeding time (Ivy) 2½ min. (normal 2-4 min.).

Coagulation time (Lee and White) 26 min. (normal 4-10 min.).

One stage clotting time (Quick) 14 seconds, control 14 seconds.

Prothrombin consumption index 100% (normal below 40%).

Fibrinogen—normal.

Anti-thrombin level—normal.

Serum electrophoresis—slight increase in alpha 2 globulin.

Serum bilirubin, proteins and non-protein nitrogen—normal.

Thromboplastin Generation Test:

Normal Serum Normal $Al(OH)_3$ treated plasma. Normal platelets.		Patient's Serum Normal $Al(OH)_3$ treated plasma. Normal platelets.	
Tube No.	Clotting Time in sec.	Tube No.	Clotting Time in secs.
1	58	1	70
2	42	2	40
3	17	3	35
4	12	4	29
5	12	5	31
6	13	6	30
		7	29
		8	31

Patient's $Al(OH)_3$ treated plasma.
Normal Serum.
Normal Platelets

Tube No.	Clotting Time in secs.
1	85
2	83
3	75
4	60
5	57
6	53
7	58
8	62

Tests for Circulating Anticoagulants:

Coagulation time of 1 ml. of normal whole blood—4½ min.
Coagulation time of 0.8 ml. of normal whole blood plus 0.2 ml. normal saline—4¾ min.
Coagulation time of 0.8 ml. of normal whole blood plus 0.2 ml. patient's plasma—over 90 min.
Coagulation time of 0.8 ml. of normal whole blood plus 0.2 ml. patient's serum—10 min.

Treatment and subsequent course: Patient was transfused with 8 pints of blood. Haemorrhage from the bowel ceased. He was discharged after 6 weeks with a haemoglobin of 10.5 g/100 ml. He remained well for 7½ months with no bruising or haemorrhage but with coagulation times of 45 min. to 54 min. on two occasions.

At the end of this period the patient was urgently readmitted in June, 1956, with massive intraperitoneal haemorrhage. The patient was transfused but died two days later.

SUMMARY

A case of circulating anticoagulants is described in a patient with no bleeding history. Diagnosis was made on the basis of the thromboplastin generation test and the effects of the patient's plasma and serum on the clotting time of normal whole blood.

The above case was presented by this Department to the late Sir Lionel Whitby during his visit to Auckland in 1956.

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AIR HYGIENE IN THE HOSPITAL ENVIRONMENT: ITS MEASUREMENT AND IMPORTANCE

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There is at least one gloomy satisfaction that unsuccessful candidates at examinations in medicine and allied sciences can share, namely, that in 20 to 30 years' time they might just be right! For instance, the examinee of 20 years ago who had the temerity to suggest that infection was very commonly air-borne would have had the following flung at him from the writings of distinguished epidemiologists, "the burden of proof that a disease is air-borne rather than by contagion or other immediate transmission rests with those who assert that air-borne infection has been operative", or this, "the last stronghold of air-borne infection has been in relation to small pox and chicken pox", or finally this, "in hospital wards, the danger of aerial convection is to be disregarded for all practical purposes. There is no occasion for assuming the growth of pathogenic germs outside the body of infection by . . . . air or any similar theory and no such theory should be adopted unless pretty strong evidence can be brought to its support." The examiner, if inclined to be more caustic, might have added for good measure, "we have progressed since the days when Pasteur left his boiled hay infusion exposed to the air" but, further back than Pasteur, we read in medical literature the following:

". . . . for, along with the air that is drawn in, there enter mixed with it germs of contagions and, where once these have been introduced, they do not retire as easily by expiration as they entered by inspiration, for they adhere closely to the humours and organs and some even to the spirits which retreat from the image of their contrary and carry the enemy with them even to the heart."

This remarkable insight into the potentialities of air-borne infection, even to describing the trapping of bacteria by the secretions of the respiratory passages and phagocytosis of the bacteria was actually written by Fracastorius as far back as 1546. Despite the dogmatic assertions of epidemiologists of a much later date, there kept occurring those puzzling cases where infection by touch could be ruled out. People who passed by windows of wards in which sufferers from varicella and variola were being treated developed these diseases themselves. In obstetrical and burns units, cross infection kept occurring despite the lack of human carrier of the streptococcus and outbreaks of neonatal gastro-enteritis due to certain serological types of E. coli, despite the most meticulous

handling of the infants, left the protagonists of contact infection short of explanations.

Infection of operation wounds in theatres led to a great deal of heart searching in operating theatre techniques. An authority on hospital cross infection felt the inadequacy of ascribing such infection as all due to touch. The necessity, induced by war, for many to lead a troglodyte existence whether in air-raid shelters or submarines had a great deal to do with intensified research upon the problem of air-borne infection. The theory of air-borne transmission of disease gradually began to reassert itself, but the ordinary means of demonstration—the swabbing and settlement plates—were not providing sufficient evidence. Then came a variety of mechanisms for sampling large quantities of air.

The bubbler sampler had a vogue but although giving a qualitative picture of air contamination, it was unsatisfactory in respect of quantitative estimation. Plainly what was required was a machine which would give an appreciation of bacterial contamination in a plainly demonstrable form, not only in volume but in time. The air contamination of a theatre or nursery may be high, but what causes it? Wells, in America, designed his air centrifuge by which the bacteria-carrying particles in a measured volume of air were flung by centrifugal force upon the nutrient medium on the walls of a cylindrical glass container. The container was then incubated and pathogens isolated.

THE AIR SLIT SAMPLER:

Bourdillon and Lidwell, in Oxford, approached the problem from another angle and eventually brought out their air slit sampler in various sizes suited to a range of volume from ½ cu. ft. to 129 cu. ft. of air. A modification of the instrument allows for around the clock sampling—a very great advantage. Unfortunately, these later models are not yet made commercially, and the nearest we can get to them is a series of interrupted samples.

Bourdillon and Lidwell's slit sampler is estimated to trap up to 90% of the bacteria-carrying particles passing through it and the success of the instrument can be shown in the fact that the latest article in an American journal dealing with the bacterial contamination of nurseries states that the English machine was used throughout. One will agree that this speaks for itself. The essential features of the Oxford machine are as follows:

An ordinary vacuum cleaner motor exhausts the air from a chamber in which a plate of culture medium is revolving at a given speed. The surface of the medium is 2 millimeters below a slit or series of slits through which the air from the exterior

passes down as a jet directly against the medium. A constant strength of suction exerted by the vacuum cleaner motor is adjusted in the machine as bought from the maker by a very crude sleeve valve. This valve makes so much noise that it was replaced by a very ingenious valve designed by Mr W. Mahn, an instrument maker of the Wellington Public Hospital. By this means, a much more easily adjusted and noiseless regulation of the air flow can be made. We found also that the motor, in itself, was so noisy we had to enclose it in various thicknesses of hard and soft board and bed it down on sponge rubber. Even now, with the noise cut down to 20% of its former strength, it can be distracting.

With the suction left at the requisite level, we adjust the volume of air by opening out one or all of the slits above the revolving plate of culture medium. So much for air volume.

The time of running and speed of rotation is adjusted by the mercury trip switch activated by a projecting arm on the revolving turntable. By different placing of the gears about the turntable, we can vary the time of revolution from one per minute to one in five minutes.

While the motor turning the timetable is of the indication variety, i.e., brushless and giving no spark, the one of the air pump is of the ordinary brush motor type which does not permit of its use in the course of operations with explosive anaesthetics.

The culture medium employed is phenolphthalein phosphate agar (Difco blood agar base) stiffened with 1% New Zealand agar (Davis) to prevent "boiling" of the medium under the high air pressure.

Our main preoccupation has, naturally, been with the Staphylococcus, which is the chief enemy nowadays in hospital cross infection. We had done some work with the Streptococcus for which we use a crystal violet azide blood agar and also tested for E. coli contamination of air for which we used the McConkey medium. The technique and isolation of the coagulase positive staphylococci, the counting of the colonies and the discussion on techniques of phage typing have all been dealt with most efficiently elsewhere and I do not intend to comment upon them here. Nevertheless, I would draw your attention to the value, indeed the necessity, for bacteriophage typing in this work. Without this, more precise designation of the numerous types of coagulase positive staphylococci or even the enumeration of the coagulase positive organisms would be almost useless. One example is a labour ward, in which there was a high coagulase positive count. The

question of installing a ventilating mechanism was in view, but phage typing revealed the source of the staphylococci to be the operator of the machine himself.

THE PRACTICAL APPLICATIONS OF THE AIR SLIT SAMPLER:

Operating Theatre:

The air-conditioning of operating theatres aims at:

(1) The safety of the patient.

(2) The comfort and efficiency of the theatre staff.

It is with the patient's safety our main work lies. First of all, what are the standards by which we work? There are none; but Leonard Colebrook has suggested that, for long operations, e.g., pneumonectomy and craniotomy, the figure should not raise above 2-5 bacteria-carrying particles per cu. ft. and, for shorter procedures, not more than 5-20.

We have employed the machine during numerous operations in several hospitals and the findings are roughly those conforming to the above suggested levels but the levels for longer procedures are not, in our experience, attained without the use of the plenum system of ventilation.

This brings us to one interesting use of the machine; to determine when a ventilating mechanism is functioning well enough to supply a safe atmosphere. On one occasion, the machine revealed that the mechanism had been installed without proper cleaning of the ducts and air sampling before use of the theatre obviated risk to the first patent to use it. Among the causes for increased bacterial load of theatre air, the instrument has shown the danger inherent in persons coming into the theatre wearing clothing of woollen material inadequately covered with a gown.

On the whole, our findings in connection with operating theatres, although on occasions showing the present curse of hospitals—the 80/81 phage type staphylococcus—tend to indicate that infections, if they occur in theatres at all, do not do so by reason of air contamination. The human carrier, theatre techniques and sterilizing mechanisms require much more attention, since there appears to be no correlation between the staphylococci in the air sampled and the subsequent history of the patient. Work in England has shown that, in any case, the air about the patient is "dead" and samples taken elsewhere three feet away from the tube do not tell the complete story. So far, the millipore filter apparatus has not proved of value in air sampling. The air slit sampler will not allow us nearer to the operating table than 3 ft.

Obstetrical Units:

Here, the slit sampler has shown a number of findings indicating the need for very radical re-thinking of our techniques.

It has been suggested, since 1948, that our present cross infection in nurseries was due to air-borne infection and this was more than indicated by tests in 1951 when, on this occasion, it included bacteriophage typing with air sampling and, by this means, the picture was more complete. In the case of obstetrical units, we lack any standards of air hygiene apart from that suggested for "any occupied space", namely, 50 bacteria-carrying particles per cubic foot.

THE EPIDEMIOLOGY OF OBSTETRICAL UNIT STAPHYLOCOCCI CROSS INFECTIONS:

A brief word on the epidemiological facts of maternity unit sepsis may be of value. The baby is born with its body surface and anterior nares devoid of staphylococci. Within a few hours, according to English authorities, the umbilical stump is colonised and, in four days the nasopharynx show staphylococci. The mother's milk, very frequently indeed, shows coagulase positive staphylococci but only after several days does it show the same phage type as that found in the baby, from whose nasopharynx the contaminated saliva has been aspirated.

Nurses show an increasing incidence of nasal colonisation throughout their training and the staphylococci, in both the noses of nurse and baby, strongly suggest air-borne infection. We have reason to think that the primary colonisation of the baby's umbilicus is by contact and nasal carriers of the staphylococcus usually carry it on their hands. Indeed, it can permeate the whole skin's surface and percolate through the clothing. In New Zealand nurseries, however, we have not always found umbilical colonisation to precede that in the nose.

Of course, an actually infected mother or infected nurse may provide even greater facilities for contact spread.

However, neither mothers, nurses nor medical attendants provide the aerial contamination which reaches its height in nurseries during the bathing session. Frankly, when I saw the first plates from the machine after a bathing session, I suspected laboratory contamination. Counts of from 200-640 bacteria-carrying particles per cubic foot were the rule where babies were bathed together. Remember the upper suggested limit of 50 per cu. ft. for all occupied spaces. In these counts, we have found no less than 2% of the colonies of the 80/81 phage type staphylococcus. This

means that, for every 7 minutes of its bath, a baby may breathe in an 80/81 staphylococcus every other bathing session and the nurse present for a 30 minute session breathes in at least 40. Do you wonder that such a high proportion of nurses' noses become colonised with 80/81 staphylococci? We do not think of subjecting the populations to drinking water of an E. coli content of 1 per m.l. We cannot expect a baby at its lowest powers of resistance to the pyogenic cocci to breathe an atmosphere sometimes 12 times the upper acceptable limit for adults, without developing some type of cross infection. Not only this, but impure nursery air is very plainly later represented in breast abscess for the mother, with all its implications.

Since it has been shown that boils and staphylococcal skin lesions generally are often the results of auto infection, this has an important bearing on nurse health.

Origin of the Staphylococci:

Whence comes the cloud of bacteria? Stripping an infected baby's bassinet and subjecting the bedclothes, one by one, to sampling in the slit sampler showed no 80/81 staphylococci anywhere except in the pillow on which the child's head was lying. Since the baby had conjunctivitis, it was only reasonable that a few colonies were found here. We then stripped the child itself and all garments were negative; only the napkin remained to be examined, when it was found that the cultures showed abundant colonies of 80/81. Obviously, the bacteria from the early umbilical or alimentary tract colonisation are communicated to the napkins and these pollute the air.

How Do We Remedy the Situation?

The foregoing results plainly show the need for investigation into new techniques and administrative methods for handling the baby. Mechanical means of ventilation have not proved very successful when applied to old buildings. A recent addition of a standard American text book on preventive medicine is most unenthusiastic on the subject of mechanical and electrical means of altering the state of nursery aerial pollution and, when an American text book fails to strongly advocate these means, they must be of extremely doubtful value.

The present vogue for "rooming-in" may be one answer, but whatever means are employed, it is evident that we cannot continue to subject babies in nurseries to this high degree of aerial contamination.

Labour and maternity wards *per se* have not shown counts widely differing from those in theatres or general wards, although in bedmaking some of the latter wards have shown undesirable figures.

CONCLUSION:

In bringing this account of somewhat superficial observations to a close, I do not wish to suggest that the problem of baby to baby—baby to nurse—baby to mother infection is solved by merely pronouncing the word "air-borne". We have some very fundamental discrepancies to clear up first. Elek, at St. George's Hospital, London, found it took 2-8 million staphylococci, whether rubbed in or injected, to produce a lesion and these experiments were conducted with coagulase positive staphylococci of a variety of types from a variety of sources, but only 100 were needed if a stitch were inserted. We cannot think of 2-8 million organisms descending from the air on a baby's skin and only after many hours will they be found in the baby's nose. Some people are colonised with the staphylococcus, others are not. Thus, we face the old problem posed by the anti-air-borne infectionists. Do we often carry 100 staphylococci on infected fingers? Does the sebaceous covering of the baby's skin play a part? What does the stitch do? Are all carriers equally dangerous? Why, if Elek's work is valid, does an actual staphylococcal lesion in a ward prove so "catching?"

The above are various problems from a multitude of questions which arise in one's mind. They are only mentioned for you to consider yourselves. Recently it was stated, apologetically, to a visiting London authority on hospital cross infection that the National Health Institute was playing around with the slit sampler and staphylococci in general. His reply was, "It doesn't matter who does the investigation as long as investigation is being done".

The foregoing is perhaps more a summary of impressions than of actual conclusions, a reconnoitre of possible routes to commence the real climb rather than assured paths to the summit. While throughout the laboratories of this country there is that continual examination of swabs for staphylococci and the production of antibiograms, what might be otherwise drudgery can be relieved by each bacteriologist turning over some of the many problems in his or her mind and pooling the knowledge so obtained.

ACKNOWLEDGMENTS

I am indebted to Dr. J. Cairney, Director-General of Health, for permission to publish this paper and Mrs J. Lange, National Health Institute, for the large amount of bacteriological work involved.

RECENT ADVANCES IN PRACTICAL HAEMATOLOGY

DR. M. McKELLAR

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I propose in this paper to limit my remarks to a discussion under selected headings of some of the recent advances in laboratory practice in haematology and to pass on a few observations made during a period of post-graduate study leave spent recently in Great Britain.

EXAMINATION OF THE PERIPHERAL BLOOD:

In the hospitals I worked in or visited in Great Britain there was a strong tendency to move away from screening peripheral blood by means of a red cell count, haemoglobin, white cell count and differential count. Instead the haemoglobin alone was estimated and more time was devoted to a careful examination of all the formed elements in the film. A general idea of the number of white cells and the proportion of different white cells present was obtained and actual counts were carried out only if some departure from the normal was seen. The film was carefully screened, particularly along the sides and in the tail for abnormal white cells and nucleated red cells. The presence or absence of anisocytosis and poikilocytosis was noted and, when the latter was present, even though involving only a small percentage of the red cells, the nature of the predominant type of deformity was commented upon. In particular, burr cells, of uraemia, carcinoma of the stomach and bleeding peptic ulcer (Schwartz and Motto, 1949; Aherne, 1957); fragmentation forms, of certain renal disorders and drug sensitivity (Allison, 1957); triangular and antler cells, of chronic renal disease (Dacie, 1954); tear drop poikilocytes, of myelofibrosis with myeloid metaplasia (Korst, Clatanoff and Schilling, 1956); and target cells, of liver disease, dehydration, postsplenectomy (Crosby, 1952) and haemoglobinopathies were specifically looked for. Target cells of no pathological significance may also be seen in normal blood films made from blood obtained by finger prick, owing to contamination of the blood by sweat (Prentice, 1957).

In a number of English hospitals the majority of screening examinations on films were done by the medical staff. In a number of hospitals, too, blood films covered with mounting fluid and coverslip were examined by a one-seventh inch dry objective lens instead of the usual one-twelfth inch oil immersion lens. Certainly anisocytosis and poikilocytosis seemed to be detected more readily with the one-seventh inch dry lens. In addition to dispensing with

the use of immersion oil, the covered films faded less readily on storage.

Recent work (Daland, Gottlieb, Wallerstein, Castle, 1956) has shown that films made from ear lobe blood may give much more information than films made simultaneously from venous blood or finger prick blood. In bacterial endocarditis in particular, not only in the ear lobe blood may the total white count be much higher, but histiocytes may be present in quite large numbers while absent or nearly so in venous or finger prick blood. Daland and her co-workers felt that many abnormal cells; probably because of their larger size than the normal formed elements of the blood, were entrapped more readily in the capillaries of the ear lobe than those of the fingers. Examination of ear lobe blood was recommended in pancytopenic states when no abnormal cells were found in the peripheral blood.

Reticulocyte Preparations:

Preliminary screening of reticulocyte preparations for Heinz bodies should not be neglected, for the discovery of Heinz bodies may be of diagnostic importance in patients with unusual haemolytic disease, in patients developing an anaemia while on drug therapy (note the Heinz body anaemia associated with salazopyrin and dapsone treatment—Spriggs, Smith, Griffiths and Truelove, 1958) and in anaemias of infancy and of the newborn, particularly when congenital defects (Allison, 1957) are present. In these preparations Heinz bodies should not be confused with Howell-Jolly bodies.

Falsely high or low reticulocyte counts as a result of overkeen or defective vision of the examiner is now well appreciated. The discovery of a practical quantitative chemical method of estimating the number of young cells delivered by the marrow into the peripheral blood would eliminate this error. An estimation of the relative number of young cells in the circulation is provided by the red cell cholinesterase level but this technique is too complicated for routine use.

Red Cell Counting and Red Cell Indices:

Red cell counts were being done infrequently in English hospitals and consequently indices, apart from the mean cell haemoglobin concentration, rarely estimated. It seems certain that many of these laboratories would undertake red cell counting and estimation of indices if entirely satisfactory and accurate red cell counting machines were available.

Whilst in Great Britain I had the opportunity to see two moving stage erythrocyte counting machines, one of American

and the other of English make, in operation. Considering the accuracy of the final results and the time taken by the machines to perform the counts both appeared to be inferior to an English electronic erythrocyte counter operating on a flow principle. I was informed that the chief disadvantage of this counter was the tendency for narrow openings in the fluid circulating system to become blocked by debris. Recently an electronic counter has appeared on the American market utilising the flow principle, but in which the counting is based not on photoelectric scanning but on the measurement of current changes due to differences in electrical resistance between the red cells and fluid diluent, as diluted blood passes through a minute electronically activated orifice.

Satisfactory microhaematocrit centrifuges have become available recently. Their quickness of action and the relatively small quantity of blood required for the test will almost certainly result in their replacing the centrifuges and methods currently used for haematocrit estimation. Results obtained by the microhaematocrit method compare very favourably with those obtained by the usual apparatus and, when dealing with blood having a high packed cell volume, the microhaematocrit technique may in fact give more accurate results.

BONE MARROW ASPIRATION FILMS:

Wherever possible one film from each bone marrow aspiration should be stained for iron. In the stained film coarse iron positive granules within the cytoplasm of reticulum cells, indicative of adequate storage iron (Beutler, Robson and Buttenwieser, 1958) and very finely granular iron positive particles within the cytoplasm of normoblasts (Kaplan, Zuelzer and Mouriquand, 1954), indicative of normal haemoglobinisation of these cells, should be looked for. The iron positive particles may be increased, decreased, absent or normal within the reticulum cells or the normoblasts. The recent observation (Beutler, Robson and Buttenwieser, 1958) that the serum iron may not fall in an iron deficiency anaemia until the haemoglobin is less than 9 or 10 grams and that deficiency of marrow iron stores precedes the development of iron deficiency anaemia, has indicated that examination of the marrow iron content alone may give more help than the serum iron and latent iron binding capacity estimations alone. It also appears likely that certain patterns of iron content within the reticulum cells and normoblasts may be diagnostic of certain diseases, particularly within the groups of hypochromic and refractory anaemias.

It is necessary to control all iron staining by simultaneously staining a control marrow known to contain adequate amounts of iron, e.g., the marrow of pernicious anaemia or aplastic anaemia.

With the well established observation that certain leukaemias respond best to certain forms of therapy, the necessity for determining the nature of a leukaemic process is obvious. However, even in the most renowned haematological centres difficulties are experienced on occasions in deciding the pedigree of leukaemic cells, whether certain blast cells are lymphoblasts, myeloblasts or monoblasts, even with the help of peroxidase staining and nucleolar examination by Feulgen staining.

INVESTIGATION OF ABNORMALITIES OF THE CLOTTING MECHANISM:

From the original publications it was not easy to see how the two-stage method of prothrombin assay (Biggs & Douglas, 1953) and the thromboplastin generation test could be carried out by a single person without help. One day of working in Dr. Bigg's laboratory showed me that both these techniques as well as the method of assaying anti-haemophilic globulin used there (Biggs, 1957) could be performed by one person, not only without help but with time to spare during the testing. The apparatus required included a series of stop watches, a thin-walled and rimmed narrow clotting tube ("Anchor" Brand, Code T. 227, Gallenkamp) and a good supply of homemade pipettes, drawn from glass with thin but robust delivery ends and calibrated by mercury to deliver 0.1 ml. of material. A fresh pipette was used for each incubation mixture. In addition to delivering aliquots of incubation mixture into substrate tubes, the pipette was used to dispose of clots formed within the incubation mixture, thus dispensing with the use of applicator sticks for removing clots after withdrawing the pipette from the tube. For instance, in the thromboplastin generation test for a few seconds before removing each sample from the incubation mixture, the pipette was wound around the inside of the tube and plasma squeezed from any clot which had formed around the end of the pipette over the preceding 50-55 seconds. The squeezed-out clot which was wrapped around the end of the pipette was left there without harm and removed at a more convenient time later.

In estimating the clotting time within substrate tubes after the addition of aliquots of incubation mixture the substrate tube was removed periodically from the water bath and, after touching a pledget of cotton wool affixed to the tube rack, to remove water

from the end of the tube, the tube flicked to throw down into an easily observable compact mass any clot which had been forming. This technique of throwing down the clot was thought to give sharper end points and dispensed with wire loops for detecting clotting, the use of which becomes cumbersome when multiple test systems are being examined simultaneously, as in the A.H.G. assay. The estimation of clotting by observing the formation of a solid clot on merely tilting the tube, is unsatisfactory in thromboplastin generation and related tests for not infrequently, particularly when haemophilic blood is being tested, solid clots fail to form.

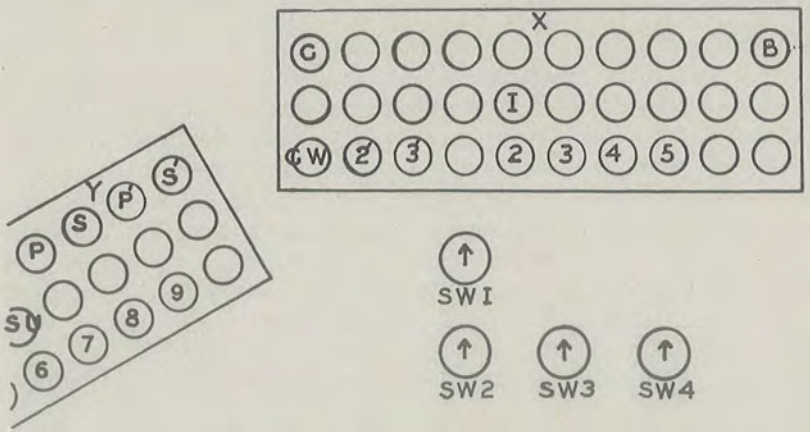

Fig. 1—Suggested lay-out of reagents and stop watches for thromboplastin generation test.

X Rack in 37°C water bath. C Calcium chloride solution.
Y Rack at room temperature. B Brain or platelet suspension.
I Incubation mixture. SU Substrate plasma.
2-5, 6-9 Substrate tubes containing substrate plasma.
2′ Position of substrate tube 2 after addition of incubation mixture and calcium chloride.
3′ Position of substrate tube 3 after addition of incubation mixture and calcium chloride.
P Patient's adsorbed plasma, diluted. P′ Control adsorbed plasma, diluted.
S Patient's serum, diluted. S′ Control serum, diluted.
SW2-SW4 Substrate stop-watches.
SW1 Master stop-watch.
CW Cottonwool pledget.

In Fig. 1 a satisfactory arrangement of tubes and stop watches for carrying out a thromboplastin generation test is illustrated. In performing a test the master stop watch is started at the moment the incubation mixture is recalcified. At approximately 50 seconds later the required 0.1 ml. quantities of calcium chloride solution and incubation mixture are drawn up into pipettes held in the left and right hands respectively. At approximately 60 seconds by the master watch the two reagents are discharged simultaneously into the first substrate tube. Without returning the pipettes to their respective tubes, the left hand still holding the calcium chloride pipette starts the "first substrate watch" to time clot formation in the first substrate tube. The two pipettes are now returned to their respective tubes where they will remain till used in a like manner some 45 seconds later. With a little practice the interval of time between the delivery of the reagents in each substrate tube and the starting of each substrate watch will be constant for all tests. Three substrate watches are usually sufficient even when there is marked overlapping of clotting times in all the substrate tubes as when haemophilic plasma is being examined.

While it is possible to carry out the thromboplastin generation test with the aid of one watch alone, the use of multiple watches is necessary when A.H.G. assays are carried out in the manner recommended by Dr. Biggs (Biggs, 1957).

The replacement of normal platelets by substitutes possessing platelet-like activity cuts down the labour associated with the preparation of the reagents in the investigation of haemophilioid disorders by the thromboplastin generation test. Chloroform extract of brain (Biggs and MacFarlane, 1957) is an easily prepared and satisfactory substitute. In A.H.G. assays a platelet substitute is almost indispensable, Folch's phospholipid extract of brain being perhaps the best substitute (Biggs, 1957).

The development of a simple screening test of thromboplastin generation by Hicks and Pitney (1957), a much more rapidly performed test than the complete thromboplastin generation test, has been an important advance in the rapid detection of abnormalities of blood thromboplastin formation (except, however, when the defective thromboplastin generation is the result of defective platelet activity). The test, although only a screening test, is as sensitive, if not more so, as the complete thromboplastin generation test in detecting disturbances of blood thromboplastin formation from deficiencies of A.H.G., Christmas factor, P.T.A. and circulating anticoagulants inhibiting thromboplastin generation.

Laboratory Control of Anticoagulant Therapy:

While most hospitals in Great Britain still control anticoagulant therapy with dicoumarol and related drugs by the Quick one-stage prothrombin time test, Owren's P and P (prothrombin and proconvertin) method for estimating hypoprothrombinaemia (Allington, 1958) is being used quite widely. The main advantage of this method over Quick's method is in its increased sensitivity to changes in the overall concentration of prothrombin and prothrombin conversion accelerators in the patient's plasma. Accurate assays of prothrombin and proconvertin activity within the 40-100% range can be obtained by Owren's method. This accuracy within this range, while particularly useful in the selection of patients for liver biopsy, is not so necessary, however, in the control of anticoagulant therapy.

In a number of European and Scandinavian centres an overall test of the clotting mechanism, the heparin tolerance time, is used in the control of anticoagulant therapy. A modification of this test has been used in America (Rosenthal and Weaver, 1952). The use of this type of test seems particularly valid in view of the effect, recognised within recent years of anticoagulants on the earlier stages of thromboplastin generation as well as on prothrombin conversion as estimated by tests of Quick and Owren.

In the preparation of acetone-extracted brain for use in the Quick prothrombin time estimation, the main precautions in the experience of the Oxford people are to avoid the use of blenders and to avoid grinding the brain too finely. Both blended brain and brain ground too finely by hand result in unsatisfactory preparations. In Dr. Biggs' laboratory the brain was ground with mortar and pestle, in usually three changes of acetone, for ¼ to ½ hour but no longer. By this time the rubbery consistency of the brain on grinding had just disappeared. Drying was carried out overnight and the dried brain was stored at room temperature in corked but non-evacuated bottles. Brain stored under these conditions was fully active several months later.

General Points in Clotting Tests:

Glassware, including pipettes, contaminated by commercially prepared thrombin should be rigorously excluded from the clotting room. Commercial thrombin, unlike natural thrombin, has a marked avidity for glassware and is very resistant to removal or destruction.

Silicone vapour may also cause trouble in clotting rooms, particularly when tubes used for estimating whole blood clotting times and prothrombin consumption become exposed to the vapour.

It is, for a similar reason, important also not to wash siliconed and non-siliconed tubes together.

Defibrination Syndrome:

In a number of cases of defibrination syndrome ("afibrinogenemia") of pregnancy, a discrepancy between the quantity of fibrinogen estimated quantitatively by the usual thrombin clotting method and semiquantitatively by its precipitation from plasma by an equal volume of half-saturated ammonium sulphate solution (Biggs and MacFarlane, 1957), has been noted. The most probable explanation of this occurrence of false low fibrinogen levels estimated by the former method is that the circulating fibrinogen has been converted to a form which cannot be clotted by commercial thrombin. It seems desirable in investigating all future cases of suspected defibrination syndrome, whether associated with pregnancy or not, to estimate fibrinogen by both methods.

BLOOD GROUP SEROLOGY:

At a conference on cross-matching which I attended at Sheffield it was unanimously agreed that adequate cross-matching should include cross-match in saline at room temperature, an albumin cross-match at 37°C and an indirect Coombs' test cross-match. There was no unanimity of opinion as to how long the tests should be incubated before reading, but most haematologists favoured one hour. Dr. P. L. Mollison pointed out that virtually all clinically significant incompatibilities were detected after 15 minutes' incubation. A panel of 10 internationally renowned haematologists felt that the albumin cross-match was an essential cross-matching test and each authority instanced several experiences of Rhesus group incompatibilities undetected by the indirect Coombs' compatibility test and detected only by the albumin cross-match. It was realised that the three cross-matching tests mentioned above would not detect all incompatibilities, even serious ones. For instance, serious incompatibility due to anti-Jk^a, an antibody fortunately of rare occurrance, is frequently only detected by using an enzyme-treated cell plus an indirect Coombs' compatibility test.

Of serological significance has been the recent experimental production of haemolytic transfusion reactions in subjects whose sera, although free from antibodies by the usual tests at the time of transfusion, had previously contained Rhesus or other antibodies.

A phenomenon of importance in cross-matching, and which should not be forgotten when investigating obscure transfusion reactions, is the deterioration of anti-Kell and, to a lesser extent,

other rare antibodies in serum during storage at low temperatures. Loss of antibody activity from a stored serum may mask frank incompatibility when this serum is used for cross-matching purposes. Deterioration of the reactivity of red cell antigens during storage, particularly the Kell antigen (Race and Sanger, 1958) may also mask incompatible cross matches on very rare occasions.

The importance of the Coombs' titration technique when using the direct Coombs' test for diagnostic purposes needs no emphasis for use of a single dilution of Coombs' reagent will not detect all warm and cold antibodies. It is important to remember that a direct Coombs' test cannot be carried out on blood which has been allowed to cool below 37°C prior to testing, for once this has occurred frequent false positive tests, although usually weakly so, will occur through auto-sensitisation of the cooled cells by naturally occurring incomplete cold antibody. Under these conditions an indirect Coombs' test and not the direct test is being performed since sensitisation of the red cells has taken place only after the blood has been removed from the circulation.

Not infrequently one is faced with the problem of providing blood for a patient with a positive direct Coombs' test suffering from autoimmune haemolytic disease. Unless particular precautions are taken during the cross-matching, a transfusion may not only be a waste of time and blood but possibly dangerous even though the blood given is apparently compatible by the usual tests. The only blood which can be safely transfused is that which is compatible not only with the patient's serum but also, since free antibody may not be present in the serum, with an antibody-containing eluate prepared from the patient's red cells. In the majority of cases of autoimmune haemolytic disease, taking these precautions in cross-matching, it is impossible to obtain completely compatible blood since the auto-antibody is most often a pan-antibody. If blood must be given when there is no completely compatible blood available, the least incompatible blood should be given (Dacie, 1954).

Rhesus Typing:

Many hospitals in England D-type their blood by two methods, the two tests being performed and read independently by two technicians. A wide range of typing methods were used including the sandwich technique of Stratton (1955). In the last two years a further method of D-typing has become available, the activated-papain technique of Löw (Löw, 1955; Race and Sanger, 1958). This method has all the advantages of a technique using enzyme-treated red cells, yet is rapid and typing serum containing incom-

plete anti-D of low titre may be used. Once-washed red cells are mixed with the activated-papain solution and the antiserum, and the whole mixture then incubated for 30 minutes at 37°C and the result read macroscopically. No washing of the red cells following enzyme treatment is required.

This technique is proving most useful in antibody screening and specificity-determination tests although, as with other techniques using enzyme-treated red cells, its use is limited by its ability to destroy or alter the reactivity of certain red-cell antigens.

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FINAL EXAMINATION FOR THE CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

August, 1958

NATIONAL HEALTH INSTITUTE, WELLINGTON

WRITTEN EXAMINATION

Tuesday, August 26, 1958

Time allowed: 3 hours. Five questions; all to be attempted.

1. What is meant by the term "alkali reserve"? Give a brief account of a method of estimation of the alkali reserve of blood plasma or serum. Mention in a few words, some of the conditions which are associated with abnormal levels.
2. Write brief notes on:—
   (a) pH.
   (b) Buffers.
   (c) Normal solutions.
   (d) The method of conversion to mEq/L of concentrations of elements or compounds expressed as mg/100ml.
3. What features in a blood film might suggest the presence of a haemolytic anaemia? Give an account of how such an anaemia is investigated in the laboratory.
4. Discuss the laboratory methods available for the confirmaton of the clinical diagnosis of diphtheria.
5. Write an account of the main features of Antibody.

PRACTICAL EXAMINATION I

Tuesday, August 26, 1958

Time allowed: 3 hours

BACTERIOLOGY

(First Session)

1. Report on this specimen.
2. The two specimens of sputum provided have been delivered to a laboratory with a request for "examination". Both specimens came from males aged 60-70 years. Make the immediate examinations that might provide useful information and indicate other or subsequent appropriate examinations.
3. Make sketches and notes illustrating—
   (a) the structures, functions and names of the parts of fungi;
   (b) the structures and life cycle of *either*
      (1) T. echinococcus
      or (2) Oxyuris vermicularis.
4. Identify the organism in culture A. The organism was obtained from faeces.
   (1) Pleural fluid containing pus and blood, enterococci and B. proteus.
   (2) (a) T.B. and commensals,
      (b) Commensal bacteria only.
   (4) Shigella sonnei.

HAEMATOLOGY

(First Session)

1. The three blood samples provided are—
   (a) an unattached pilot tube;
   (b) a specimen taken without contamination from the corresponding bottle;
   (c) a pretransfusion sample from the patient.

There has been a reaction to this blood. Make all the appropriate examinations, so far as you are able, and comment.

(1) No cause for the reaction was demonstrable in the specimens provided.

PRACTICAL EXAMINATION II

Wednesday, August 27, 1958

Time allowed: 3 hours

BACTERIOLOGY

(First Session)

Complete the identification of organisms in questions 1 and 4 from the previous day.

HAEMATOLOGY

(Second Session)

2. Carry out the following examinations on blood specimen "E":
   (a) Reticulocyte count;
   (b) Make and stain films and report on these.
3. Examine blood films 1 to 6 and write a report on each.

The following were the six slides given in Haematology question 3.

1. Basophilic stippling.
2. 56% Eosinophils.
3. Acute myelogenous leukaemia.
4. Glandular fever.
5. Normal blood.
6. L. E. cells.

Wednesday, August 27, 1958

PRACTICAL EXAMINATION III

BIOCHEMISTRY

Time allowed: 3 hours

1. Outline a method of estimation of the serum inorganic phosphate, and carry out the estimation on serum sample A provided.
   Describe briefly the errors which are likely to be associated with each stage of the estimation, and make a rough estimate of the probable accuracy of the final result.
2. Urine sample B is from a patient who may be suffering from acute pancreatitis. Describe the method which you would use to estimate the concentration of urinary diastase, and carry out the estimation on the sample provided.
3. The blood and urine specimens C provided have been obtained from a Urea Clearance test carried out on a patient. Carry out urea determinations on each of the specimens, indicating the method which you use, and work out the urea clearance for this patient.
   Mention briefly the factors which are of importance in carrying out a good urea clearance test on a patient.
4. (a) Test the urine sample D provided for bilirubin.
   (b) Determine whether the urobilinogen content of urine sample E is within normal limits, or is elevated.
   (c) Sample F is a solution in 0.4% ammonia of haemoglobin from an unconscious patient who it is suspected has been exposed to coal gas. Examine this with the hand spectroscope, and compare it with sample G which is normal control solution of haemoglobin. Is there a difference? If so, what is its character and what does it indicate?

SUCCESSFUL CANDIDATES—

Miss E. M. TITTERINGTON (Wellington).

Miss M. R. EMANUEL (Auckland).

Miss Y. YOUNG (Napier).
Miss P. TINDALE (Timaru).
Mr C. S. O. SHEPHERD (Hamilton).
Six candidates sat the examination.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

November, 1958
NATIONAL HEALTH INSTITUTE, WELLINGTON
Examiners: Dr. W. L. Kenealy, Mr M. O. Ekdahl.
Wednesday, November 5, 1958

WRITTEN PAPER

Time allowed: 3 hours—9.30 a.m. to 12.30 p.m.
All questions carry equal marks.

1. Write a concise account of the procedure you would follow in examining a specimen of urine for M. tuberculosis.
   Give details of the composition of stains and medium used.
2. What organisms are commonly associated with outbreaks of food poisoning?
   Describe laboratory methods used in the investigation of such outbreaks.
3. You are asked to determine the coagulation time, bleeding time and platelet count of an adult.
   Give, in detail, the method you would use in each instance and state the normal values for the methods you employ.
   For what technical reasons is the normal range of a platelet count so wide?
4. Give the principle (technical details of these tests are *NOT* required) underlying any one method of determining—
   (a) Blood sugar;
   (b) C.S.F. chloride;
   (c) Faecal occult blood. Explain why it may be desirable to reduce the sensitivity of this test. How is this done?
5. Write brief notes on the following:
   (a) The significance of haemolysis in bacteriological practice;
   (b) Temperature control of laboratory apparatus;
   (c) Disposal of infected material;
   (d) The use of filters in microscopy.

PRACTICAL PAPER

Wednesday, November 5, 1958, 2.30 p.m. to 5.30 p.m.
and
Thursday, November 6, 1958, 9.30 a.m. onwards.
Question 1 to be completed on November 6, 1958, and Question 6 to be done on November 6, 1958.

1. Identify, as far as possible, the organisms provided in pure cultures A and B—
   (a) Sh. flexner;
   (b) S. enteriditis.
2. You are provided with serum C from a patient for whom a blood transfusion is required, together with samples of blood from prospective donors D. E and F—
   (a) Group the donors;
   (b) Select and cross-match a suitable donor.

3. Make a routine examination of the urine supplied. (Culture *NOT* required.
4. Determine the sugar content of the C.S.F. provided (the standard contains 100 mg./100 ml.).
5. Perform a red cell count, leucocyte count and differential count on the blood sample provided.
   State briefly the essential details of the steps you take.
6. Report briefly on the "spots" provided.
   (a) McIntosh and Fildes Jar.
   (b) Incubator capsule.
   (c) Lymphatic leukaemia smear.
   (d) P.A. smear.
   (e) Smear of a sporing bacillus.
   (f) Constant volume pipette.

In the Oral Examination the candidates were questioned on the following:—

McIntosh and Fildes' jars; constant volume pipettes for media; reticulocyte count methods; the staining reaction of reticulocytes; hot air ovens; checks of sterilisation procedure; principle of centrifuge and precautions in use; incubator capsules; composition of stains; composition of media; Certificate of Accuracy accompanying Grade A glassware.

SUCCESSFUL CANDIDATES:—

Miss R. J. PAINE (Gisborne).
Miss C. M. McKENZIE (Invercargill).
Miss B. M. SLATER (Dannevirke).
Miss S. E. COXHEAD (Auckland).
Miss S. A. FURKERT (Auckland).
Mr K. A. WATTS (Auckland).
Miss J. M. CHRISTENSEN (Christchurch).
Miss C. A. CURRIE (Hastings).
Miss F. M. BLACK (Wellington).
Miss G. M. STAIRMAND (Wellington).
Miss M. A. MIDDLEWEEK (Wellington).

JUNIOR ESSAY COMPETITION

Entries for this competition close with the Editor on June 20th, 1959. Entrants must state for which section they wish to enter and give their name and address on a separate piece of paper. All trainees are eligible for this competition.

TECHNICAL SECTION:

Descriptions of methods or techniques in use in the Laboratory.

ESSAY SECTION:

Essays on historical or general aspects of Laboratory work.

A prize of £5/5/- is offered for the best entry in each section.

SUBSCRIPTIONS

Members are reminded that subscriptions to the Association for the year ending 31st March, 1959, are payable now to the Treasurer.

c/o Mr M. McL. Donnell,
Hon. Secretary,
N.Z. Association of Bacteriologists,
17 Anzac Street,
Takapuna,
AUCKLAND.

Seniors: £1/10/- Juniors: 15/-

NEW ZEALAND HAEMOPHILIA SOCIETY (INC.)

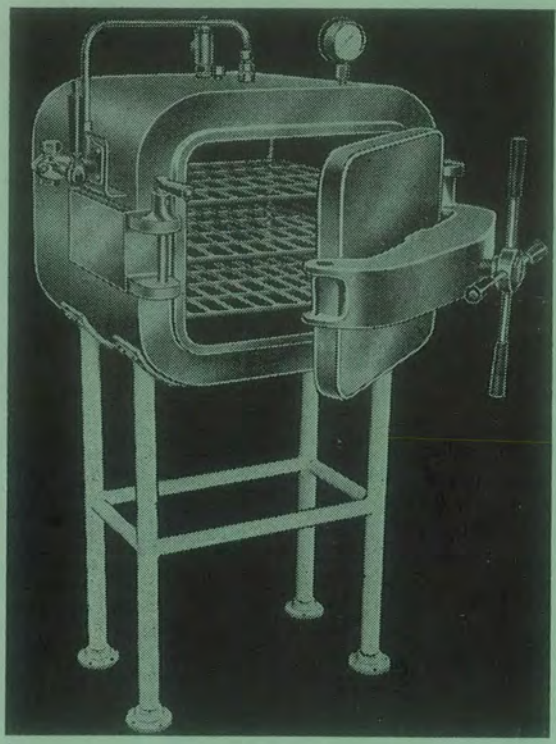
The New Zealand Haemophilia Society was formed in May, 1958, and the aims and objects of the organisation are similar to Haemophilia Societies in overseas countries.

We have been asked by Mr R. D. Jones, Chairman of the Council, to draw your attention to the existence of the Society and to publish the following information.

The aims and objects of the Society are as follows:

1. To list all haemophiliacs and Christmas disease sufferers and compile family histories and other data which may be of assistance to the general medical profession.
2. To support research, inquire into all new methods of treatment, cures and alleviations of the haemophilic problem.
3. To assist in the education, vocational guidance, rehabilitation and social adjustment of sufferers from haemophilia and to generally assist the haemophiliac to become a useful and productive member of the society.
4. To act generally as a friendly and charitable organisation for the benefit of haemophiliacs or sufferers with Christmas disease.
5. Assisting voluntary blood donor banks to obtain donors.

For any further information or offers of help, please contact Mr R. D. Jones, P.O. Box 105, Lower Hutt, Wellington.



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