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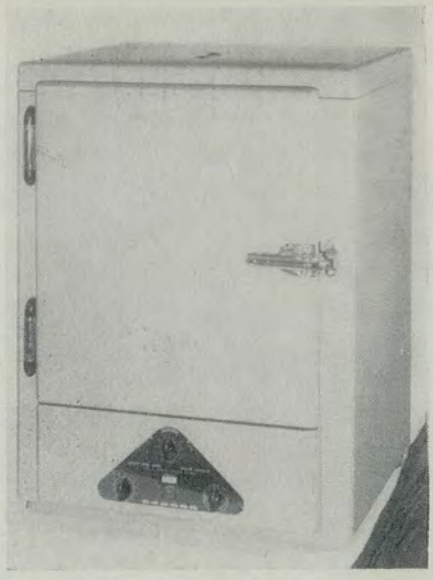

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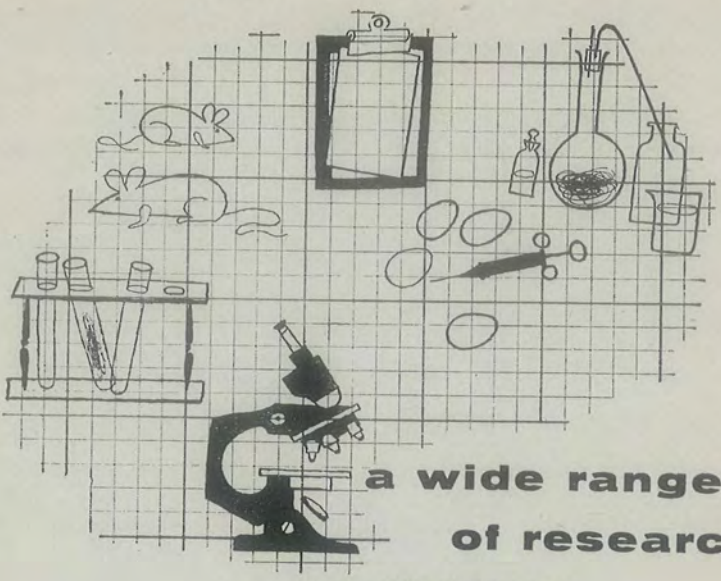

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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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ERRATUM:

Vol. **12,** 3, 1957 ("Diagnosis of Haemorrhagic Disorders", R. Kennedy).
p. 64, Heparin (+ CO factor found in albumin fraction).
p. 68, References.
Pietro de Nicola.
Dameshek and Stefanini.

THE HAEMOLYSIN SCREEN TEST FOR DETECTION OF IMMUNE ISO-ANTIBODIES IN UNIVERSAL DONORS

Miss L. EVANS

(*Pathology Department, Christchurch Hospital*)

GENERAL

The universal use of group O donors, generally a safe and acceptable procedure, is not without some danger as blood transfusion reactions have resulted from the usage of the universal donor. There is now a great deal of evidence that red cell destruction caused by transfusion of a group O donor to a recipient of another group depends mainly on the presence of an "immune" type of anti-A and anti-B antibody.

An association exists between the levels of naturally-occurring and potent immune iso-antibodies, but a high titre of an immune iso-antibody may exist in the presence of a relatively low level titre of a naturally-occurring iso-antibody. Group specific substances may be added to group O blood to neutralize the naturally-occurring iso-antibodies but neutralization of the immune iso-antibodies remains ineffective. Furthermore a group O recipient of blood containing group specific substances may develop immune types of iso-antibodies that render the plasma of such an individual dangerous for use at a subsequent period. In a female recipient this procedure may lead to disease of the newborn where ABO incompatibility exists. It thus emerges that the saline agglutination titre of plasma is not a reliable guide to its power of causing red cell destruction *in vivo* and secondly that the addition of group specific substance to group O blood does not necessarily make it incapable of causing red cell destruction.

Thus the problem arises in practice, how to identify those group O donors whose blood would be dangerous if used for recipients in other groups, because of the character of its antibodies. This problem has been attacked in various ways, as estimating the titre of the antibodies, but none has, as yet, proved reliable. In this paper a test is described which has been suggested by Gardiner and Tovey for screening donors, and which relies on the demonstration of anti-A and anti-B haemolysin, the rationale being that iso-haemolysis is one of the characteristics of immune antibodies. It is probable that, like other tests which have been used, the haemolysin test over-estimates the incidence of "dangerous" universal donors. This is of little importance in normal hospital work, but might become significant under wartime conditions if only group O donors were to be used.

CHARACTERISTICS OF IMMUNE ANTIBODIES

1. Act as haemolysin in conjunction with complement.
2. Fix complement.
3. Increase in titre by using a medium of high colloidal concentration (such as AB serum) as a diluent.
4. React in the indirect Coombs' test after partial neutralization of the naturally-occurring iso-antibodies in the serum.
5. React more strongly at 37°C than 4°C.

STIMULATION OF IMMUNE ISO-ANTIBODIES

Immune iso-antibodies develop in the sera of most group O people receiving one of the following stimuli:

1. T.A.B. vaccine.
2. anti-tetanus serum.
3. injection of human A or B substance.
4. injection of animal AB-like substance.

In anti-tetanus serum the source of the A-like substance is in all probability the pepsin derived from the hog-stomach and used for digesting the horse serum. (Pepsin from hog-stomach is a known source of A-like substance.) In the T. A. B. vaccine the source of stimuli may be the Forssman-type antigen which is known to be present in many bacteria, but may be present in the medium on which the bacteria are grown.

MATERIAL FOR TEST

1. *Donor serum:* blood specimens are stored at 4°C. To minimize loss of complement, the serum should be separated immediately prior to testing and within 24 hours after collection. Plasma should not be used, as anticoagulants are anticomplementary.

2. *Red cell suspensions:* cells from single strongly reacting group A_1, and B donors are used. These are washed and made up to a 2% physiological saline suspension. Group O cells 2% suspension are used for control tubes.

3. *Control sera:* sera containing anti-A haemolysin and anti-B haemolysin are used. These are obtained from previously tested individuals showing such reactions. If stored in sealed tubes and kept frozen solid in CO_2, the haemolytic activity will remain for about two months. Human complement must be added to these before the test.

4. *Apparatus:* glass tubes 12 by 75mm. and rack to hold three rows of tubes.

As this test may be incorporated in the routine grouping of donors, the same racks and tubes are used providing there is an extra row for tubes which contain controls.

TEST PROCEDURE

1. Add 1 drop donor's serum into each of the three tubes.
2. To tube 1 add 1 drop group A_1, red cell suspension 2%.
3. To tube 2 add 1 drop group B red cell suspension 2%.
4. To tube 3 add 1 drop group O red cell suspension 2%. This third row is used as a control for comparison.

Two control tubes, one containing anti-A and the other anti-B control sera, should be included in the performance of each day's test, and to these tubes A and B red cell suspensions are added respectively, also fresh sources of complement.

5. *ALL test and control tubes are immediately incubated at 37°C for one hour.*
6. After one hour's incubation, using a good source of light, preferably daylight, and with minimum disturbance, examine tubes in rows 1 and 2 for any haemolysis, comparing these against row 3 which is the negative control.
7. Classification of haemolysis:—

bloods showing complete	haemolysis	+ + + +
bloods showing 75%	„	+ + +
bloods showing 50%	„	+ +
bloods showing 25%	„	+
bloods showing trace	„	tr.
bloods showing no trace	„	O

From this classification haemolysis detected can be divided into two groups, "strong" embracing + + + +, + + +, and + +; and "weak" embracing + and trace.

8. "Strongly" haemolytic bloods are regarded as potentially dangerous for universal use and should be labelled appropriately to be used for group O recipients only. Tubes showing "weak" haemolysis or which are negative are regarded as safe.

TEST VARIABLES

1. *Storage of test specimens:* storage for more than 24 hours at 4°C results in detectable loss of activity due to decline of complement; if specimens are more than 24 hours old an equal volume of fresh human serum free from lysins can be added as source of complement.

2. *Age of test cells:* storage for more than 2-3 days does not appear to have significant effect on the reactivity of the cells.

3. *Red cell variation:* variation in resistance to haemolysis of red cells of different individuals appears to be minimal.

4. *Divalent cations:* apparently the divalent cation contribution of the test serum is sufficient for optimum activity. Divalent cations are required for optimum activity in immune antibodies.

5. *Red cell concentration variation:* a primary consideration in performing haemolysin screening procedures is the selection of a suspension of cells of such a concentration that the full haemolytic reactivity of the test serum is obtained with the amount of complement that is present in the reaction mixture. 2% cell suspensions appear to give most satisfactory results, suboptimal amounts of complement may be present in tests in which the suspension of cells has a concentration greater than 2%.

6. *Prozone reactions:* these have not been encountered.

RESULTS

No. Group O donors bled 1955/56	6163
No. showing haemolysis 1955/56	1045
=	16.5%
Showing anti-A haemolysis	89%
Showing anti-B haemolysis	4%
Showing anti-A and B haemolysis	7%

DISCUSSION

An alternative procedure of allowing the mixture to stand at room temperature for 1 hour followed by 1 hour's incubation at 37°C gives a test of slightly lowered sensitivity, probably as a result of greater interference with haemolytic activity by the naturally-occurring iso-antibodies. Initial incubation minimizes this.

It is desirable that the red cell suspension is no higher than 2%, as it is indicated that greater numbers of cells lead to suppression of the haemolytic reaction.

Examining tubes for the presence of haemolysis in bright daylight is found to make the reading much easier as haemolysis may not be discerned when fluorescent lighting is used as a light source.

The incidence and level of anti-A haemolysins are shown to be considerably greater than those of anti-B. This higher incidence is consistent with the higher incidence commonly demonstrated for anti-A iso-antibody.

This test described here and used in many laboratories is one based on a haemolysin test as a screening procedure for "immune" iso-antibodies as the presence of these "immune" iso-antibodies in universal donors has been demonstrated to be the cause of some haemolytic transfusion reactions. Although it is convenient to divide the antibodies of the ABO blood group system into naturally occurring and immune, it must be realised

that this distinction is not absolute, and there is need for a more comprehensive study of the kinetics of anti-A and anti-B haemolytic reactions.

Furthermore, as pointed out in the introduction, the precise significance which should be attached to the findings of haemolysis, is not yet known. For this reason it is probably best to regard all group O donors with haemolysis as potentially dangerous to other groups, until the relationship between the laboratory test and the tendency to give rise to clinical haemolytic reactions has been clarified.

SUMMARY

Among the various characteristics of immune antibodies the ability to lyse red cells is the easiest to detect. Therefore a routine haemolysin test is described and suggested as a screening procedure for group O donors whereby potentially dangerous universal donors will be recognised. The test is simple, and can be incorporated in the ABO grouping of donors.

ACKNOWLEDGEMENT

I wish to thank Dr. F. W. Gunz for his helpful criticisms and advice in the preparation of this paper.

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SOME TECHNICAL ASPECTS OF THE ERYTHROCYTE SEDIMENTATION RATE IN DISEASE AND HEALTH

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(Winner, Essay Section, Junior Essay Competition)

When discussing the estimation of the erythrocyte sedimentation rate (E.S.R.), we must remember that medical practitioners use this test as a screen for, or confirmation of, suspected organic disease, and then as a means of assessing progress of such disease.

It is known that there are many factors contained in the blood, and technical factors, which can cause results to be far from accurate. The presence of a pathological condition normally shown by a high E.S.R. may be hidden, or on the other hand, a high E.S.R. may be due to a relatively unimportant, but nevertheless contributory point. I shall try to account for this statement in the following paragraphs. First, however, we must attempt to understand how and why the red cells fall, and what influences the rate of fall in organic disease.

In the sedimentation of red cells there are three phases. The first (following the setting up of the test) is a time of aggregation, during which the cells clump in rouleaux formation. The second phase is the fall of these clumps through the plasma, and the third is the slow packing of the settled cells.

It is recognised that organic disease causes changes in the plasma proteins, which accelerate the formation of rouleaux and the sedimentation rate is correspondingly hastened. Plasma with raised fibrinogen, or raised globulin levels, show raised E.S.R.s. Any increase in size of the protein molecules, as found in macroglobulinaemia, has the same effect. There have been various attempts to account for this.

Whitby and Britton (1953) state that the red cells carry a negative and the plasma a positive electrical charge, this causing the rouleaux to form, and any increase in the plasma's positive charge hastening the process.

Eastham (1954), however, states that there is a direct relationship between plasma viscosity, and the E.S.R. He supports this theory by showing that in the presence of substances which increase the viscosity, but do not affect the proteins, the E.S.R. is raised. When fibrinogen and globulin values are increased the viscosity is correspondingly increased. It is interesting to note that he says the raised viscosity hastens rouleaux formation, but retards the fall, the former overriding the latter in effect until a viscosity of 2.65 (relative to water) is reached, when the action is reversed and the effect of the latter predominates. It is a pity that this statement is not upheld with examples of the first phase being hastened and the second retarded.

It is known that the plasma proteins fall progressively during pregnancy but rapidly rise to normal during the first month post partum. The E.S.R. follows this course, being raised throughout and quickly resuming normal levels. This does not help clarify the protein-E.S.R. relationship. Pregnancy and menstruation are the only physiologically normal conditions in which a raised E.S.R. is found.

Now that we have an idea of what happens in the sedimentation of erythrocytes, we must consider the factors influencing, and the errors possible in, the test. This must not only cover technical points, but also those actually contained in the blood itself.

The plasma is known to carry a few factors which may lead to an E.S.R. result not relevant to the state of disease. An increased quantity of albumin or lecithin is quoted as retarding the E.S.R. (Whitby & Britton). Some drugs will temporarily reduce the E.S.R. and when treatment is discontinued there is a rapid return to the raised sedimentation rate, which is not due to any change in the diseased state. "Butazolidin," A.C.T.H., mercury compounds, and intensive sodium salicylate therapy act this way. A.C.T.H. will reduce the rate even in normal people. Sulphonamide treatment may increase the E.S.R. temporarily. Occasionally a falsely low E.S.R. is obtained a few days after a patient has been treated with a therapeutic antitoxic serum. A day or two later the previous level is resumed. This action is not understood. The effect of abnormalities in the serum proteins has recently been demonstrated by two cases seen in Green Lane Hospital.

The first was a case of macroglobulinaemia found in a man who was relatively well, but had an E.S.R. of 110 mm. His total serum protein was 8.9 gm.%, albumin 2.4 gm.% and globulin 6.5 gm.%. The haemoglobin was 10.2 gm.%.

In contrast there was a case of agammaglobulinaemia. A young man at the height of a severe attack of pneumonia showed an E.S.R. of 2mm. His total serum protein was 4.6 gm.%, albumin 3.4 gm.% and globulin 1.2 gm.% and haemoglobin 16.0 gm.%. A marked lowering of the E.S.R. is characteristically seen with agammaglobulinaemia.

Probably the biggest source of error in interpretation of the E.S.R. is caused by alterations of the red cell-plasma ratio. This does not apply in the same way when a liquid anticoagulant or solid sodium citrate is used—as will be shown later. The Heller-Paul anticoagulant (potassium and ammonium oxalate) as used in the Auckland Hospital Board laboratories was used in the following examples, and the discussion applies to the method incorporating this anticoagulant, the 200 mm Westergren tube (2.5 mm bore) and a one hour reading.

Lawrence (1953) investigated the effects of different cell-plasma ratios on the E.S.R. He found that bloods with a packed cell volume (P.C.V.) with any significant variation from the average normal of 45% often had an E.S.R. result not consistent with the patient's condition or diseased state. Of nineteen cases studied, each with a P.C.V. of 48% or over, one had an E.S.R. of 25 mm and the remaining eighteen had E.S.R.s of 13 mm or less. The P.C.V.s of these bloods were adjusted to 45% and E.S.R.s set up. Six of these now showed a fall of over 30 mm. The results obtained this way were consistent with the patients' respective conditions. As examples I quote cases A and B:—

Case A. Pulmonary tuberculosis; P.C.V. 56%, E.S.R. 7 mm. When the P.C.V. was adjusted to 45% the E.S.R. became 61 mm.

Case B. Polycythaemia vera; P.C.V. 59%, E.S.R. 1 mm. The blood with adjusted P.C.V. showed an E.S.R. of 3 mm.

The difference between these two examples was due to the fact that Case A was organic disease, whereas Case B was not.

On the other hand it was found that any blood with a P.C.V. of 41% or under, when adjusted to 45%, had a lower E.S.R. I quote Cases C and D as examples.

Case C. Hypochromic anaemia; P.C.V. 27%, E.S.R. 41 mm. When the P.C.V. was adjusted to 45% the E.S.R. became 7 mm.

Case D. Carcinoma of the pancreas; P.C.V. 36%, E.S.R. 93 mm. On the blood with adjusted P.C.V. the E.S.R. was reduced to 73 mm.

Thus we can see that the adjustment of P.C.V. in some cases will show a critical difference in E.S.R.. A routine haemaglobin done with all E.S.R.s is usually considered sufficient indication of the P.C.V. to show the value of the test.

The size of the red cells has some effect on the E.S.R.—microcytes sediment more slowly, and macrocytes more rapidly than normocytes. Macrocytes larger than 130 cu. microns, however, reverse the process and sediment slowly (Lawrence).

Whitby & Britton state that by interchange experiments with cells and plasma of normal and iron deficient blood, it has been proved that a factor retarding the sedimentation of iron deficient blood lay in the cells themselves. This supports Lawrence's idea that cells with a low colour index sediment slowly.

The question of the use of oxygenated or deoxygenated blood for E.S.R. estimation really arises here, but I will deal with it further on.

There are left now, many technical factors to consider, in the estimation of the E.S.R. To begin with it is vital to have one standard method adhered to in all respects so that results are reproduceable.

Temperature variation can cause appreciable differences in E.S.R.s and for this reason Todd (1946) suggests that the E.S.R. racks be stood in a 37° C. incubator, and the readings taken at the end of half an hour. He says that the results are almost identical with the one hour reading at 18° C. Possible errors due to cold agglutinins which can cause falsely high E.S.R.s are avoided, and the convenience of the reduced time is obvious. The only drawback I can see to this idea is that an error of two or three minutes would be magnified, and could cause an important difference.

The height, width and inclination of the tube used, affects the E.S.R. result. The height of 200 mm with a 2.5 mm bore has been found preferable. The shorter Wintrobe tube accentuates the buffering action of the cells, similar to that found with a high P.C.V.

As far as I know, the vertical position of the tubes has always been used. Although I have been unable to find any literature about it, I have heard that there are experiments being carried out with the tubes held at a definite angle. It may well be that this would, to some extent, overcome the problem of varying P.C.V., as the buffering action would be reduced. The time lapse would probably be much reduced also.

The reflux factor is the term applied to a phenomenon encountered with different methods of filling the tubes used in the test. If blood is drawn above, and then allowed to fall back to the O mark, a higher result is obtained than if a sucker is attached to the tube, and the blood drawn to, and stopped at, the O mark. For some reason this factor affects the first, or aggregation, phase, hastening it if reflux is allowed.

Deoxygenated blood is an occasional source of error. The presence of carbon dioxide inhibits rouleaux formation, and slows the fall. Two factors seem to influence this. In deoxygenated blood the erythrocytes are slightly swollen and the P.C.V. correspondingly higher than in oxygenated blood. Duxbury (1954) found that the P.C.V. of deoxygenated blood could be as much as 6% higher than when the same blood was fully oxygenated. Also if the cells are slightly swollen with the carrying of carbon dioxide they must be verging on a spherocytic shape, and spherocytes do not readily form rouleaux. Provided venous blood is inverted gently for four or five minutes after taking, it becomes oxygenated enough to prevent any real error. The time lapse between taking blood from the

patient, and setting up the test, should be minimal. The sooner the test is set up, the better. After two hours the blood seems to lose its power of forming rouleaux, and the E.S.R. markedly decreases. It is pointless to use blood after this time. Although it is possible that some method may be devised to enable accurate tests to be carried out at long intervals after collection, such techniques are not generally available at present.

Finally I will discuss some anticoagulants in use. Solid sodium citrate is not advised. It makes the plasma hypertonic, causing osmosis and a consequent loss of fluid from cells to plasma. Diluted plasma and shrunken cells result, the P.C.V. is lowered, and the resultant changed blood is unsuitable for other haematological investigation.

However, when isotonic sodium citrate solution is used, one part to four parts of blood, the osmosis does not occur. The addition of the fluid does, nevertheless, result in a lowered P.C.V. and a diluted plasma. The reduced P.C.V., as previously shown, hastens the fall of the cells, but the diluted plasma means that the plasma sedimentation rate (S.R.) factors are weakened and these two effects do not balance. According to Lewi and Clarke (1954) when the plasma S.R. factors are normal, or only slightly raised, their dilution almost obliterates their effect, and even coupled with the reduced P.C.V. this results in a lowered E.S.R. However when the plasma S.R. factors are raised the dilution is not sufficient to make any great difference and the E.S.R. obtained is higher than on undiluted blood. Apart from the difficulties encountered in working with a fluid anticoagulant which necessitates accurate measurements, there is, I think, one big failing. Early diagnosis plays a most important part in the curing of many organic diseases. Any method of estimating the E.S.R. which hides the first signs of such disease, must have big advantages in other ways to be justifiably used.

Heparin as an anticoagulant seems to share the same chief disadvantage as the potassium and ammonium oxalate mixture, this being that a raised P.C.V. in conjunction with organic disease will rarely give a raised E.S.R.

SUMMARY

The E.S.R. is a reflection of the balance of protein fractions in the blood, and will indicate degrees of disturbance of this balance which occurs in various diseased states.

Certain technical factors can influence the E.S.R., and cause a false reading. These include temperature variations, height, width, and angle of tube, time allowed for sedimentation, the de-

oxygenated state of venous blood, and the time elapsing between collection of the specimen and carrying out of the test.

Provided physicians are familiar with the method in use in their laboratory and the possible errors of the method, the E.S.R. is a simple and very useful test.

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DIAGNOSIS OF HAEMORRHAGIC DISORDERS II

R. KENNEDY

THROMBOPLASTIN GENERATION SCREEN TEST (Hicks and Pitney 1957)

Principle

The test plasma is diluted with veronal buffer and recalcified in the presence of a platelet substitute. The thromboplastin thus generated in this incubation tube is measured by adding samples along with calcium chloride to normal substrate plasma at 60 second intervals and recording the clotting times. Minimum clotting times are reached in 3 to 5 minutes with normal plasma.

The thromboplastin generation test (Biggs & Douglas 1953 (a)) is at the moment the key test for differentiating deficiencies of the thromboplastin forming substances. The test, however, suffers in that it is too time consuming for routine screening of suspected haemorrhagic disorders. Indeed we have found that to prepare reagents and carry out a full thromboplastin generation test, more than four hours' work is entailed. The advent of a platelet substitute (Bell & Alton 1954) has made the performance of this test somewhat easier but still very time consuming for routine use.

The introduction of the thromboplastin generation screen test, has therefore filled a much needed gap in routine screening of haemorrhagic disorders, and while it makes no pretence at being a specific diagnostic aid, it is a most useful addition to the battery of screening tests.

THE THROMBOPLASTIN GENERATION TEST (Biggs and Douglas 1953 (a))

Principle

At least five components are required for thromboplastin formation. They are:—

(1) Antihaemophilic globulin.
(2) Christmas factor.
(3) Factor V.
(4) Factor VII.
(5) Platelets and calcium ions.

These substances are prepared and incubated together at 37°C. Thromboplastin is generated and can be tested for by adding samples with excess calcium at intervals to normal substrate plasma and noting the clotting times.

Reagents

A set of reagents is prepared from the patient and from a normal subject.

$Ca\ Cl_2$—0.025 M.

Platelets. Platelet substitute (Bell & Alton 1954).

Antihaemophilic Globulin and Factor V. These are prepared by adding aluminium hydroxide to citrated plasma. The alumina has the property of adsorbing from the plasma, Christmas factor and Factor VII thus leaving antihaemophilic globulin and Factor V.

Christmas Factor and Factor VII. Serum provides a source of these substances, as unlike antihaemophilic globulin and Factor V, neither are consumed during coagulation.

Summary of Method:

Deficiencies of Factor V and VII are excluded by the one stage clotting time. (Kennedy 1957.)

The substances we are therefore concerned with are antihaemophilic globulin and Christmas factor.

The normal set of reagents is first tested and minimum clotting times of 9-12 seconds should be produced in 4-6 minutes.

The patient's set of reagents are then tested and if the minimum clotting times exceed the control it must be determined which of the patient's reagents, i.e. alumina, plasma or serum, is at fault.

The procedure is to mix the reagents and test them again first using the patient's adsorbed plasma in place of the normal plasma and then using the patient's serum in place of the normal serum. In this way diagnosis of haemophilic or Christmas disease can be made.

In rare cases of qualitative platelet deficiency the patient's platelets can be found to give an impaired thromboplastin generation. For diagnosis in these cases platelet suspensions have to be prepared instead of using platelet substitute.

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THE FINDING OF MIMEAE IN ROUTINE LABORATORY WORK

R. W. BARRINGTON
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INTRODUCTION

How often in routine bacteriology is one tempted to "identify" an organism from colonial appearance alone? Under pressure of work the temptation may be great, yet even allowing that the antibiotic sensitivity of an organism is of greater clinical importance than its exact nature, do we not risk grave errors, and at the same time deprive ourselves of interest by such a hasty technique? The chance of finding something a little out of the ordinary should be one of the attractions of bateriological work, as witness the following case:—

An elderly patient had for years suffered from a chronic bronchitis for which he had on several occasions been admitted to hospital. In 1954 records reveal a sputum culture showing a heavy growth of "Neisseria". On his latest admission in 1956 a further specimen of sputum was sent to the laboratory for culture. The specimen, mucopurulent and light green in colour was cultured on nutrient agar containing 5% citrated human blood. Discs for penicillin and aureomycin sensitivity were added. After 18 hours aerobic incubation at 37°C. there was a heavy almost pure growth of colonies which ranged in size from 1-2 mm. and showed a zone of complete beta-haemolysis extending for an average diameter of 6 mm. The organism was insensitive to penicillin and sensitive to aureomycin. White, smooth and convex, regular in outline and possessing a slight sheen, the colonies resembled those of beta-haemolytic staphylococci *and at this stage were almost reported as such.* However, the colonies showed a mucoid consistency when touched with platinum loop and a Gram-stained film showed many Gram-negative diplococci resembling Neisseria species but the presence of an occasional bacillary form was noted. Further search of the film revealed several fusiform shapes varying between 7 and 20 microns in length as well as a small proportion of straight bacillary forms. At this stage the ratio of diplococcoid/bacillary forms was about 100/1. The organism was then suspected of belonging to the tribe Mimeae described by De Bord (2) and the following investigations were carried out.

LABORATORY INVESTIGATIONS

After replating to ensure purity, the organism was tested as follows:—

Glucose, lactose, saccharose and mannite (Difco media)

showed no fermentation after aerobic incubation for 5 days at 37°C. On Kligler iron agar the organism grew poorly producing mainly bacillary forms. Loeffler's medium was slightly softened and both coccoid and bacillary forms were noted in equal proportions. In tryptose phosphate broth (Difco) plus 0.1% agar the growth was luxuriant, being first a hanging surface growth without pellicle and after 48 hours a uniform turbidity with slight deposit. The presence of capsules could be demonstrated by a wet preparation of India ink. The organism was non-motile and non-sporing. Indole and methyl red tests proved negative.

A culture was forwarded to the Communicable Disease Centre, Georgia, U.S.A., and subsequently identified as belonging to the tribe Mimeae and the genus Herellea. This identification was based on serological and biochemical tests which showed acid and gas production in certain carbohydrates (including several that had not been fermented during preliminary tests here). The reason for the disparity in fermentation was not at first clear, but the author considers it to be probably due to differences in the base used for the media. The Americans routinely use both a synthetic base (4) and heart-infusion broth (Difco) for their carbohydrates when testing Mimeae and a regular and consistent difference between the two is noted (see table). The Wairoa strain was unusual in that it was beta-haemolytic and S.S. positive.

TABLE SHOWING FERMENTATION REACTIONS OF MIMA POLYMORPHA IN CARBOHYDRATES WITH A DIFFERENT BASE

Carbohydrate	Synthetic Base	Heart Inf. Broth (Difco)
Glucose	A	—ve
Xylose	A (rare—ve)	—ve
Mannitol	—ve	—ve
Lactose	—ve (or late A)	—ve
Sucrose	—ve	—ve
Maltose	—ve (occ. A)	—ve

A = Acid Production.

DISCUSSION

Since the original culture of Herellea two other cultures belonging to the tribe Mimeae have been isolated at Wairoa. One of the genus Colloides was isolated from C.S.F. and appeared to be the causative organism of meningeal symptoms; the other, Mima polymorpha, was isolated in pure culture from a urinary infection. In all cases the patients responded well to antibiotic therapy.

The question arises whether it is worth while in routine work to differentiate Mimeae from organisms such as Neisseria catarrhalis which it superficially resembles. A glance through the small but mounting literature leaves little doubt as to the importance of so doing. Mimeae have been found in conditions varying from therapy-resistant gonorrhoea (1) to meningitis (3) and even fulminating septicaemia (5). Moreover, in C.S.F. the microscopic appearance may be identical with that of meningococci and upon culture from either of these, colonies of Mimeae that appear will probably resemble N. catarrhalis, and if few in number may finally be ignored as "contaminants". The possibilities for mistaken diagnosis are obvious, but fortunately being aware of these possible errors is being more than half-way to avoiding them. Any suspicious colonies appearing on solid media should be subcultured into fluid media in which the proportion of bacillary/diplococcoid forms will increase to 50/50 or more.

SUMMARY

The findings of one example of each of the three genera of the tribe Mimeae at a small hospital laboratory is described with particular reference to the genus Herellea. The need for the differentiation of Mimeae from other organisms is stressed.

ACKNOWLEDGEMENTS

The author is indebted to the Staff of the Communicable Disease Centre, Georgia, U.S.A., for their work on the culture submitted and for information on fermentation reactions, etc. Thanks are also expressed to Mr H. D. Erlam, Librarian at the University of Otago, for providing microfilms.

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A ROUTINE METHOD OF TESTING SENSITIVITY TO ANTIBIOTICS

H. C. W. SHOTT

(Department of Microbiology, Medical School, Dunedin)

INTRODUCTION

The testing of bacterial sensitivities against an increasing range of antibiotics has made it essential that a simple and rapid method be devised. Morley (1945), Copeland (1945), Kokko (1947), Bondi et al (1947), advocate the use of filter paper discs. The purpose of this work is not to study in detail the various methods of performing such tests, or to examine all the factors which influence the results but to provide a modification which will give uniform results in the hands of all workers.

Practical Considerations.

1. A method which deals with the bulk of the daily routine sensitivity tests should not be regarded as a substitute for discs being applied directly to a primary culture plate where the findings in the Gram stained film indicate such a measure.
2. It should be confined to pathogenic bacterial strains which have been isolated from a mixed growth or would yield only a scanty growth on media after first incubation.
3. In order to produce uniform results the potency of each disc employed should be adequately controlled.

MATERIALS AND METHOD

1. Preparation of Broth Cultures

Representative colonies are picked from the platings of strains for test and inoculated into 2.5 ml. quantities of nutrient broth. The Oxford H. strain of Staphylococcus pyogenes (NCTC. 6571) is employed as the control organism and a broth culture prepared in the same way. After about 5 hours incubation at 37°C. the sensitivity plates are inoculated from the resulting growth.

2. Choice of media

Nutrient agar, poured in large petri dishes to the approximate depth of 3 mm. (Hayes 1945) are used for staphylococci and Enterobacteriaceae, the appropriate medium being employed where more fastidious organisms are encountered.

3. Inoculation of Plates.

Fig. 1 is introduced to illustrate the application of discs. The following technique is employed.

A sufficient volume of the incubated broth suspension of organisms *under test* is delivered at the inner periphery of the agar to spread in an even film 15 mm. around the edge of the plate. (Usually 2 drops, approx. 0.04 ml. is sufficient.) This is accomplished by the use of a glass spreader which is held in

the right hand whilst the left rotates the plate in an anticlockwise direction. By this means an even distribution of bacteria is ensured.

The discs are then applied with the aid of sterile forceps, being arranged at the inner edge of the inoculated area. Finally a loopful of the control Oxford staphylococcus is seeded from the centre of the plate up to the inner edge of each disc. The plates are incubated at 37°C.

4. Reporting the Results of Sensitivity Tests

After overnight incubation the reading is made by comparing the inhibition zone of the unknown organism with the standard staphylococcus.

SUMMARY

The method employed not only allows a comparison to be made between the inhibition of the "test" and standard strain by a given antibiotic but the potency of each individual disc is ensured.

The test complies with the observations of (1) Garrod (1945, 1948) and (2) Ungar, D (1951).

(1) The action of antibiotics was greatest on rapidly multiplying organisms.

(2) Discs should be placed not less than 15 mm. from the edge of the plate.

ACKNOWLEDGEMENTS

I wish to thank Professor J. A. R. Miles for permission to publish this paper and Dr. N. P. Markham for his encouragement and advice.

The photographs were taken by members of the Medical School Photographic Department and the glass spreaders made by Mr E. Facer, of the Glass-blowing Department, to whom I express my thanks.

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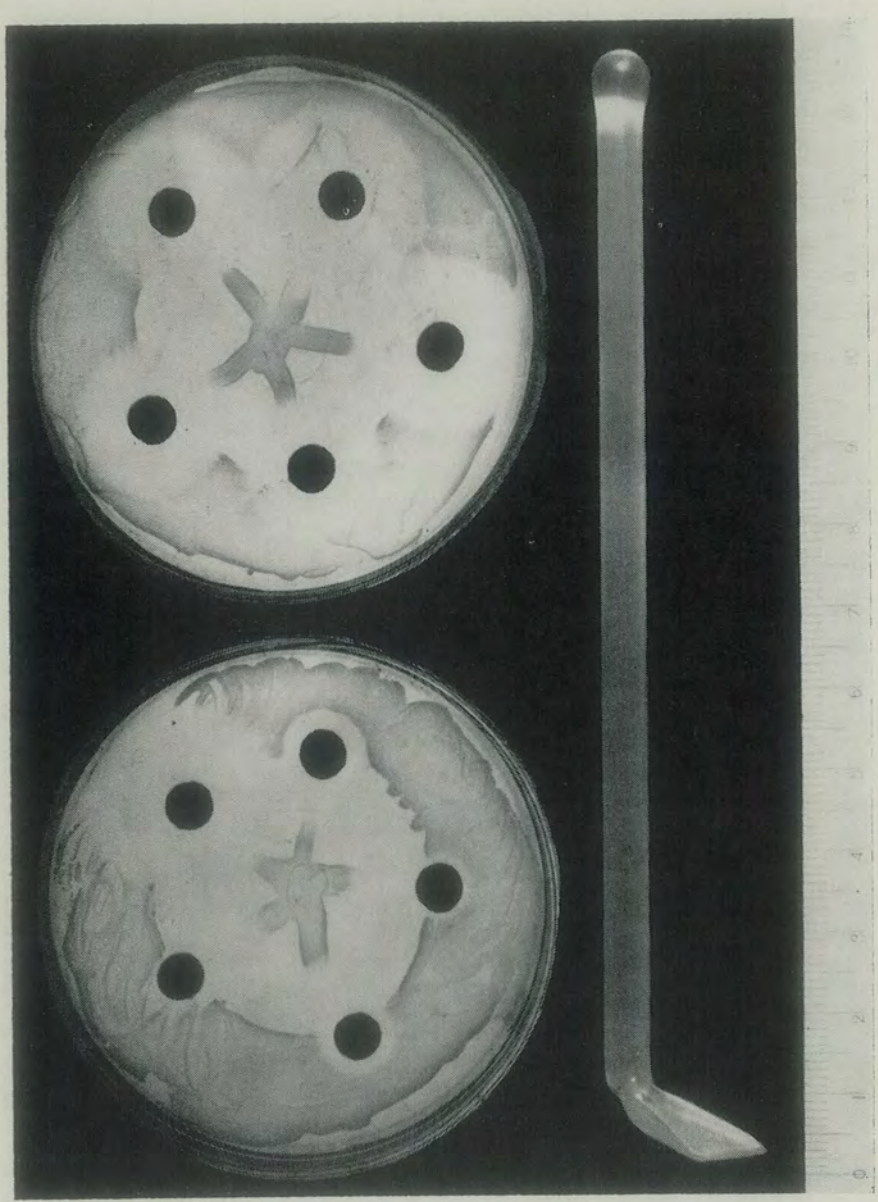

Fig. 1

THE COAGULASE PHENOMENON

I. C. KING

(Pathological Laboratory—5 O'Rorke Street, Auckland)

(Present address—Central Laboratory, Auckland Hospital)

A characteristic property of pathogenic staphylococci is their ability to produce the enzyme coagulase, which has the power to clot human and many animal plasma. Coagulase is produced only by pathogenic staphylococci, of human or animal origin and for this reason a test for it is a valuable *in vitro* criterion of pathogenicity. (Cruickshank 1937.) The theory is that the staphylococcus liberates a "prostaphylocoagulase" which acts on a fraction of the plasma globulin to produce an active enzyme. (Kaplin & Spink 1948.) The probable mechanism by which a coagulase positive staphylococcus attains pathogenicity lies in its ability to form a plasma clot around the site of the lesion (Menkin 1935), producing a highly effective barrier against the phagocytic leucocytes which would permit the body defenses to control and eliminate the infection; this would also explain why the majority of pathological staphylococcal lesions remain localized. However, Menkin and Walston (1935), indicate that the rapid obstruction of lympathic channels is produced by necrotoxin and not by coagulase.

The enzyme coagulase is thermostable, frequently antigenic and filterable; the presence of coagulase may be readily demonstrated in sterile filtrates of cultures in fluid medium provided the latter contains plasma. Coagulase activity may also be detected directly in purulent material even when there is a mixed infection or too few staphylococci to demonstrate microscopically. False negative results have been obtained from mixed or contaminated broth cultures but these appear to be due primarily to Gram negative bacilli and non-haemolytic streptococci. Fermentable carbohydrate in the medium should be avoided since Neter (1937) has shown that under these conditions many strains form an anti-coagulase which may inhibit the clotting of plasma.

TUBE COAGULASE TEST

This is the method devised by Fisk (1940). 1 ml. of a 1:10 dilution of human citrated plasma in physiological saline is mixed with 5 drops of an 8 to 18 hour broth culture and incubated for 3 hours at 37°C. Observations should be made every hour but clotting should occur after 1 hour. Since plasma may undergo spontaneous coagulation a control tube containing plasma diluted with saline should always be put up, as well as tubes inoculated with a known coagulase positive and a known coagulase negative strain.

Recent observations have shown that heparin-plasma is to be preferred to citrated-plasma in order to avoid the risk of false positives due to clotting being produced by citrate-utilizing organisms. (Harper & Conway 1948.)

TUBE CULTURE METHOD

5 ml. of broth, not digest, is inoculated with a single colony of staphylococci and 10 drops of human plasma is added, the tube being incubated for from 8 to 18 hours at 37°C. It should be noted that the relative concentrations of plasma and broth culture are moderately critical and poor clotting is often obtained if the plasma concentration is greater, or the culture concentration much less, than those given.

Heparin must be added to the citrated blood as in the tube coagulase method. Calcium in the medium will combine with the citrate also, thus permitting false clotting of the plasma.

SLIDE COAGULASE TEST

A single staphylococcal colony, or part of a colony if large, is emulsified in a drop of physiological saline on a clean slide. One drop of citrated plasma is added and the two well mixed; in a positive test clumping of the cocci takes place within 5 to 10 seconds. Negative results must be checked by the tube method.

PLATE COAGULASE TEST

Agar plates containing 25% human plasma are inoculated from a single colony of staphylococci and incubated 8 to 18 hours at 37°C. Coagulase positive strains are surrounded by a halo in the medium but these should be checked by the tube method for false positives. (Williams & Harper, 1946.)

SLIDE COAGULASE TEST USING FIBRINOGEN

The advantages of using solutions of fibrinogen instead of plasma for carrying out the slide coagulase test were pointed out by Berger (1943) when he showed that their use avoided false positives due to naturally occurring staphylococcal agglutinins in rabbit and human plasma, in addition to the fact that these solutions retained their activity considerably longer. The method used here is the Spencer modification of Berger's method. (Spencer, 1954.)

Preparation of Crude fibrinogen

Fresh human plasma is one-fifth saturated with ammonium sulphate and precipitation is usually complete at room temperature in from 10 to 15 minutes. This precipitate is firmly thrown down by centrifuging at 2,500 r.p.m. for 5 minutes when the supernatant is poured off and the container inverted on filter paper for 5 minutes to drain. The precipitate is then taken up in 10% aqueous urea to a volume roughly the same as the volume of plasma originally taken.

For use the solution is kept in a bottle with a teated pipette drawn out to a fine tip to deliver small drops on to the slide. One colony of staphylococci is emulsified in one drop of fibrinogen with the aid of a platinum loop and the test read, microscopically if necessary, after 3 to 5 minutes in a wet chamber in the 37°C incubator.

The source of plasma is usually the centrifuged unused remains of blood from the bottom of transfusion bottles but the remains of oxalated blood from biochemistry and haematology may be used. If centrifugation is inadequate to clear plasma of debris the fluid may be passed through a Seitz filter with a clarifying pad. The solution in urea remains active for one month on the bench away from sunlight and for up to 5 months in the refrigerator.

While the tube coagulase reaction will remain the more delicate test if done under standard conditions, the slide coagulase reaction is a valuable screening one and essential when numerous strains of staphylococci may be encountered in a day's work. The fibrinogen slide method is not a screen, however, but is a definitive test and is both rapid and reliable.

False positives: Provided the tests are made as described false positives do not occur. Heavy granular growth may be mistaken for clot but will disintegrate on shaking, whereas a clot will contract but remain solid.

False negatives: If the culture is incubated longer than 20 hours the clot may lyse. Fibrinolysin producing organisms in contaminated cultures, e.g. streptococci, will give negative results.

ACKNOWLEDGEMENT

I wish to thank Mr J. E. Pery-Johnston of the Pathological Laboratory, Auckland, for his helpful advice and criticism in the preparation of this article.

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EXAMINATION FOR CERTIFICATE OF PROFICIENCY IN HOSPITAL LABORATORY PRACTICE

AUGUST, 1957

NATIONAL HEALTH INSTITUTE, WELLINGTON

Examiners: Dr. F. J. Cairns, Dr. F. W. Gunz, Dr. J. D. Manning.

WRITTEN PAPER

Monday, August 26th, 1957

Time Allowed: 3 hours.

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

1. Write a brief essay on the errors affecting blood counting, mentioning examples. Discuss the ways in which such errors can be minimised.
2. Describe in detail the technique of the Coombs' test (direct and indirect). In what circumstances may this test need to be used?
3. Discuss undulant fever, including,
   (a) method of infection.
   (b) characteristics of the organism.
   (c) methods of laboratory diagnosis.
4. How would you identify a reducing substance in the urine?
5. What is the principle of the urea clearance test? How would you estimate the blood urea, and what is the principle of the test?
6. Describe the essential components of and method of operating,
   (a) a photoelectric colorimeter.
   (b) a still.
   (c) a Seitz filter.

PRACTICAL I

Tuesday, 27th August, 1957

2.30 p.m.-5.30 p.m.

BACTERIOLOGY

1. Identify the organism in culture "A".
   (Salmonella paratyphi B.)
2. Identify the organism in culture "B".
   (Proteus morgani)
3. Examine the specimen of pus "C" for pathogenic organisms.
   Questions 1, 2 and 3 are to be completed tomorrow.
   (Micrococcus pyogenes or coagulase positive staphylococus)
4. Make and stain films of the organism in culture "D", "E", and "F" and describe their morphology, naming the group to which they belong where possible.
   "D" C. diphtheriae.
   "E" Bacillus cereus.
   "F" Brucella.
5. Examine a direct film of sputum "G" for Mycobacterium tuberculosis.

HAEMATOLOGY

6. Specimen "H" is cord blood from a new-born infant. Examine it to determine:
   (a) if it is affected by haemolytic disease.
   (b) how severely.
7. Determine the ABO and D-type of the red cells "J".
   Describe your method in detail and state how you would cross-match a bottle of suitable blood.
   (a) under routine conditions.
   (b) if you were allowed only ten minutes for cross-matching in an emergency.

PRACTICAL II

Wednesday, 28th August, 1957

9.30 a.m.-12.30 p.m.

BACTERIOLOGY

Complete the identification of organisms in cultures "A" and "B" and pus "C" as far as the time available allows.

HAEMATOLOGY

1. Carry out the following examinations on blood specimen "K".
   (a) haemoglobin estimation.
   (b) reticulocyte count.
   (c) make and stain films and write comments on their appearance.
2. Do differential leucocyte counts on the films "L", "M", and "N".
   "L" Neutrophil lecuocytosis.
   "M" Glandular fever.
   "N" Eosinophilia.
3. Examine the blood films 1 to 6 and comment on any abnormal features which you may find.
   1. Hypochromic anaemia.
   2. Pernicious anaemia.
   3. Spherocytic anaemia.
   4. Erythroblastosis foetalis.
   5. Glandular fever.
   6. Malaria—P. vivax.

PRACTICAL III

BIOCHEMISTRY

Wednesday, 28th August, 1957

2.30 p.m. to 5.30 p.m.

Give methods of tests and how you arrived at your results.

1. Estimate the level of chlorides in the sample "P" of C.S.F. provided.
2. Estimate the level of blood uric acid in the sample "Q" of oxalated blood.
3. Do a routine examination of the specimen "R" of gastric resting juice. With the sample of N. NaOH provided estimate the degree of acidity (total and free).
4. Do a Van Gieson stain on the paraffin slide and mount the stained section.
5. Read the colloidal gold test on C.S.F. How is this test performed and what are the precautions?
6. (a) Test the specimen "S" of faeces for occult blood. What is the colour change in this test due to?
   (b) Test the sample "T" of urine for acetone. What is the clinical significance of this test?

SUCCESSFUL CANDIDATES:

Mr M. G. HARPER.
Miss J. A. MAITLAND.
Miss J. A. MILLS.

INTERMEDIATE EXAMINATION FOR HOSPITAL LABORATORY TRAINEES

October, 1957

Examiners: Dr. Flora Smith, Mr H. Bloore.

THEORETICAL PAPER

October 30th, 1957. 9.30 a.m.-12.30 p.m.

1. Give an account of the food requirements of bacteria in general. Name the ingredients in the following culture media, and state why they are included in these media.
   (1) McConkey agar.
   (2) Tellurite blood agar.
   (3) Leoffler's serum.
2. Define the following terms:
   (1) Normal solution.
   (2) Titre.
   (3) Mycelium.
   and give descriptions of, and the methods of working of
   (4) a Seitz filter.
   (5) Bacteriological autoclave.
3. Describe your methods of performing cross matching tests to determine the suitability of blood for:
   (1) an urgent transfusion and
   (2) a non-urgent transfusion.
   Comment on the reasons for employing the particular methods you describe.
4. What are the chief puncture fluids sent to a laboratory? Describe how you would perform a routine examination on them.
5. Give a recognised method for each of the following:—
   (a) detecting occult blood in faeces.
   (b) testing for acetone in urine.
   (c) performing a reticulocyte count.
   (d) doing a coagulation time.

PRACTICAL PAPER

2.30 p.m.-5.30 p.m., October 30th, 1957. (Continued at 9.30 a.m. on October 31st.)

1. Estimate the chlorides (as NaCl) in the CSF provided, by direct titration, using the $AgNO_3$ and potassium chromate indicator. Standard NaCl = 585 mgm. NaCl/100 ml. supplied. Show your calculations.
2. Perform the following on the blood sample supplied:
   red cell count.
   leucocyte count.
   differential count.
   prepare and stain your own films.
3. Report briefly on the blood films provided—no counts are required.
   (a) hypochromic microcytic anaemia with normal white blood cells and differential and abundant platelets.
   (b) reduced haemoglobin and platelets and abnormal and nucleated red cells.
4. Make a complete routine examination, including culture, of the catheter urine provided.
   (Pus, R.B.C.'s staphylococci and B. coli.)
5. You are supplied with a pure culture of an organism. Broadly identify this as far as possible in the time available. (S. typhi murium).

6. Identify the organisms in the pure cultures supplied, on the basis of cultural appearances, Gram stain, and any other tests for which you have time.
(N. catarrhalis, coagulase positive staphylococcus, Ps. pyocyanea, Str. viridans.)

Complete Q. 4, 5 and 6 on Thursday

ORAL EXAMINATION

Mr Bloore:

Questions based on the written and practical papers and, in addition, the following:—

Benedict's qualitative reagent.

Detection of protein in urine, including Bence-Jones.

Detection of acetone and bile in urine.

Anaerobiosis, pH, selenite broth.

Counting chambers, CSF sugars, NPN, reasons for cloudiness on nesselerisation, gastric analysis.

Cleaning of glassware, thermostats, meaning of N.A. of a lens and its practical significance.

Dr. Smith:

Various biochemical tests as in the syllabus, first aid, postal regulation, stains, glassware, equipment, how to deal with infected material.

SUCCESSFUL CANDIDATES:

Mr C. BLACKSHAW (Whangarei).
Miss R. A. DONNELLY (Waipukurau).
Miss M. I. EDWARDS (Wellington).
Miss E. N. EGLINTON (Palmerston North).
Miss M. E. OLSON (Palmerston North).
Mr J. L. MORROW (Wellington).

JUNIOR ESSAY COMPETITION

Entries for this competition close with the Editor on June 30th, 1958. Entrants must state for which section they wish to enter and give their name and address on a separate piece of paper.

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.

ABSTRACTS

A RAPID SCREENING TEST FOR DISORDERS OF THROMBOPLASTIN GENERATION

N. D. Niche and W. R. Pitney (1957)
Brit j. Haemat., 3, 227

A rapid screening test for disorders of thromboplastin-generation is described which is as sensitive as the orthodox thromboplastin-generation test in demonstrating deficiencies of thromboplastin-precursor substance. Although it is a non-specific test, nevertheless it can be used in routine investigation of suspected coagulation disorders to eliminate further and longer coagulation studies. The principle of the test is that diluted whole plasma is recalcified in the presence of a platelet substitute (chloroform extract of acetone-dried human brain) and the thromboplastin generated is tested by adding subsamples of the incubator mixture, together with excess calcium, to normal "high spun" citrated plasma. Results obtained with the test correlated well with those of the orthodox test in a series of patients with a variety of haemorrhagic disorders.

IMPROVED L. E. CELL TEST USING FINGER-PRICK BLOOD

H. Lempert and G. M. Berlyne (1957)
Brit. med. J., 1, 1041.

A method for producing L.E. cells from finger-prick blood is described in which the substrate recommended is obtained from lymphocytes of cases of chronic lymphatic leukaemia. The method compares favourably with a reliable venous blood method and has the added advantage of simplicity and ease of microscopical examination. The substrate slides prepared from lymphocytes appear to be stable for at least three months. In patients suffering from systemic lupus erythematosus L. E. cells are found consistently by the finger-prick method prior to and during treatment. In some normal subjects and in a few cases of rheumatoid arthritis an occasional cell resembling the L. E. cell may be found but the finding is not consistent.

BLOOD CHIMERISM IN A PAIR OF TWINS

P. B. Booths, Gertrude Plaut, and J. D. James
Elizabeth W. Ikin
Phillis Moores, Ruth Sanger and R. R. Race (1957)
Brit. med. J., 1, 1456

Mixed blood in dizygotic twins is common in cattle but appears to be extremely rare in man. This paper describes some tests on the blood of a brother and sister who are twins; the blood groups show that each twin is living on red cells only, some of whose ancestors were directly inherited, the rest having been acquired as grafts "in utero" from the opposite twin; the presence of female "drumstick" nodules on the nuclei of some of the polymorphs of the male twin shows that ancestral white cells too must have been successfully grafted.

The two components of the mixed blood possessed by the twins differ in five systems: ABO, MNSs, Rh, Duffy, and Kidd. After separation of the A and O red cells by a method relatively simple in principle, further grouping with anti-sera from other blood groups is straightforward.

The male twin has 86% A and 14% O red cells, the female twin has 99% O and 1% A red cells. The A cells are MSMs, CDe/cde, Fy^bFy^b, Jk^aJk^b; and the O cells are MSMS, cDE/cde, Fy^aFy^b, Jk^aJk^a; in other groups they do not differ. Secretion tests on the saliva show that the A series belong genetically to the male twin and the O series to the female.

HUMAN BLOOD CHIMERAS (A STUDY OF SURVIVING TWINS)
J. W. Nicholas, W. J. Jenkins and W. L. Marsh (1957)
Brit. med. J.,1, 1458.

A study of binovular twins is described, each of whom has blood which is a mixture of the same two distinct types differing in ABO, MN, and Rhesus groups. A family study of the blood groups has been made. Nuclear sexing of neutrophils shows that foreign leucocytes are present in the blood of the male twin and probably present in the female twin.

THE EFFECTS OF RH. GENOTYPES ON THE SEVERITY IN HAEMOLYTIC DISEASE OF THE NEWBORN
Sheilagh Murray (1957)
Brit. j. Haemat., 3, 143

An analysis of the Rh. genotype in 540 fathers of babies with haemolytic disease of the newborn is listed. The overall series, and a particular group of cases where there have been previous stillbirth due to the disease indicate (a) an increased risk of immunization if the husband is R_2R_2, (b) an increased risk of stillbirths in the disease pattern in the family, particularly where the husband is R_2R_2. Genotypes were performed on 168 samples of cord blood from affected babies and the results related to the severity of the disease in the babies. If a newborn baby is of the genotype R_2r, the outlook is poorer than in R_1r baby, in that there is a 70% chance that a R_2r baby will require treatment compared with only a 50% chance in a R_1r baby.

CANICOLA FEVER IN MAN THROUGH CONTACT WITH INFECTED PIGS
J. D. Coghlan, J. Norval and H. E. Seiler (1957)
Brit. med. J., 1, 257

Five cases of canicola fever, diagnosed on clinical and serological evidence, is reported. These cases occurred over a five-year period among piggery workers in Edinburgh. A survey of 47 piggery workers on 12 farms showed from the results of serological examinations that 40% had at some time been infected with L. canicola. It was shown that pigs may harbour L. canicola without showing any noticeable symptoms of disease. The organisms are excreted in the urine, and infection is passed from pig to pig. Human infection may result through contact with infected pigs.

SUBSCRIPTIONS

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

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Hon. Treasurer,
N.Z. Association of Bacteriologists,
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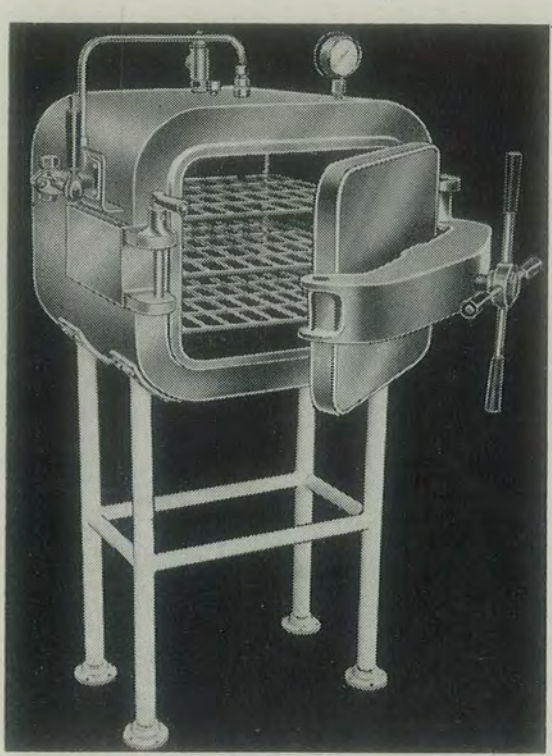

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