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Communications primarily concerning the Institute should be addressed to the Secretary.

Intending contributors to the Journal should address their communications to the Editor at the Department of Pathology, Medical School, Dunedin. Copy must be in the hands of the Journal Committee by not later than the first of the month preceding the month of publication.

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Contributions to the Journal do not necessarily reflect the views of the Editor, nor the policy of the Council of the Institute.

Our Journal — Some Changes

That something in the nature of a revolution has overtaken the Journal will have been immediately apparent when it was removed from its envelope. Some of the changes may be greeted with approbation; that others will be met with regret, with dismay, some even with frank disapproval, is unfortunately only to be expected.

It must be emphasised from the outset, that nothing we have done should be taken as implying any criticism of the previous Editors. No five people in New Zealand are more conscious than the present Journal Committee of the amount of worry and hard work that attends " being responsible for the Journal "; furthermore, it should be common knowledge that Miss Lois Evans and Gilbert Rose, not to mention others before them, have been battling for a long time to keep the Journal alive. Regrettably, we have to face the fact that the foe has been neither more nor less than the apathy and the profound indifference of the vast majority of the general membership of the Institute. These are harsh words, yet history can testify to their truth.

At the end of 1955, when the management of the Journal was passing from a committee in Auckland to one in Christchurch, A. M. Murphy[1], the then Editor had this to say: " A Journal such as this requires a change of Editor and Committee at regular intervals if it is to progress. New approaches and new ideas are constantly required." Words of wisdom indeed — and presumably it was hoped that the new committee would have greater success than its predecessor in attracting material. At first there was a little more interest taken, but in 1957, the idea of replacing the Journal with a periodical newsletter was being given serious consideration. Happily this idea was abandoned and the Journal struggled on; but the minutes of many Council Meetings since have included the sad report of the Editor—" No material."

The changes made by the present committee have been made with a view to revitalising the Journal and to creating among all members of the Institute, both senior and junior, the feeling of ownership which is so essential. There must be plenty of material available which requires only the effort of writing it up; we need this material if we are to survive, for the fact must be faced that for the continued existence of the Journal to depend upon the publication of inferior matter, is to place the prestige and reputation of the Institute itself in jeopardy.

The change of title is intended to bring us into line with other periodicals in the same field. It has the sanction of Council and few objections are anticipated since the result can only be an improvement in the standing of the Journal.

The layout has been rearranged and the list of contents relegated to the back page. The notes for guidance to would-be contributors on the inside front page, will appear in every issue. Items which have been featured in the past and subsequently dropped, have been reintroduced. These include "Abstracts" which cannot pretend to be more than a mere selection from the many articles likely to be of assistance in keeping our members up to date. With the formation in recent years of four branches of the Institute, the allocation of space to "Branch Reports" is only reasonable. The circulation of technical and scientific information remains our primary object, but items of domestic interest will be given their place as well.

Since the Journal was first printed commercially, printing costs have risen steeply and there has been a steady increase in the amount spent each year on publication. Council has voted a generous sum to cover this year's expenses and in addition, a campaign to increase the advertising revenue is having moderate success. With the size and quality of the Journal thus assured, a plea for reciprocal support of our advertisers seems justified.

The new colour is perhaps the hardest change of all to justify. In all honesty, it really represents a curiosity to see how the Journal cover would look in a new colour. However, suppose we take it as symbolic of change as a whole, and of a new era of pride in this Organ we own. If the new colour or any of the other alterations result in a deluge of protesting letters on the head of the Editor, then at least the committee will have fulfilled one important object—that of inspiring a little interest in the Institute's own publication; a commodity which has been singularly lacking in the past.—J. C.

1. Editorial in the Journal of the New Zealand Association of Bacteriologists, Volume 10, Page 39 (October, 1955).

An Introduction to the Thrombotest

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From a paper read at the 1962 Annual Conference of the N.Z.I.M.L.T.
(Received for publication July, 1962)

I propose to start with a brief outline of the factors in the blood clotting mechanism which are affected by oral anticoagulants and then to discuss the three main laboratory methods for the control of patients under treatment.

As you will know there are two systems which work independently to form blood clots—the intrinsic and the extrinsic.

The extrinsic operates when a wound is inflicted on the skin surface. Large quantities of tissue thromboplastin are released and combine with other factors in the system to form a clot in a matter of a few minutes.

The intrinsic system, on the other hand, operates inside the blood vessels themselves and it seems probable that its action starts off much more slowly than the extrinsic system, but gains in speed as it goes. This point should be remembered because factor IX which belongs to this system is not depressed suddenly, the level decreasing slowly over a period of time which allows its detection before the point of haemorrhage is reached. For example, if the skin is punctured and the clotting time is taken in a normal subject, the clot will occur in three to four minutes. If after 24 hours the scab formed over the puncture site is carefully removed and the clotting time repeated, this time will be found to be longer than the primary. This is the intrinsic system or secondary bleeding time working, and can be timed because all the tissue thromboplastin, which was previously released, has been used up in the extrinsic clot.

At the present state of knowledge it is known that four factors are depressed by oral anticoagulants. I stress the word oral as other anti-coagulants such as heparin do not work in the same way and are therefore not controlled by the tests under discussion.

The four factors are—

Factor II — prothrombin, which occurs in both systems.

Factor VII — proconvertin, found in the extrinsic system only.

Factor IX — Christmas factor, occurring only in the intrinsic system.

Factor X — Stuart-Prower factor, found in both systems.

What these factors actually do could be the subject of another paper, suffice it to say that they are the four factors to be reckoned with in the laboratory control of the patient on anticoagulant drugs plus an unknown called contact activation[8]. This latter appears to be released when the blood comes in contact with a water wettable surface, such as glass. Its effect is to shorten the clotting time if the test is not done within an hour or two of venipuncture. The closer the patient is to the normal clotting time the faster is the effect of contact activation. In normal blood it can be detected in a few minutes. It is of interest to note that this applies equally well to all three methods discussed here, *viz.*, Quick's, P & P and Thrombotest. It makes one wonder whether it has anything to do with the odd occasion when the patient's Quick test is several seconds below the normal for the day.

To overcome this we now collect the blood in plastic disposable syringes and plastic containers, as in the laboratory in which I work, we collect the blood from the patient in bed at home, which may be up to 20 miles from the base. Further, as there may be 40 or more such patients scattered around the district, it is always some hours before the blood is brought in.

So much then for the factors involved, now for the tests themselves.

For many years Quick's one-stage clotting time using thromboplastin was the accepted test to control patients on oral anticoagulants with varying degrees of success. Unfortunately there are many and varied thromboplastins on the market. Most of them are liable to be unstable not only in the entire batch but also in individual packs within a batch. The activity curves usually use saline as the diluent which dilutes all the factors present to a point where at about 50% they become inactive. If plasma is used as the diluent the therapeutic range inside which the patient should be kept, is reduced to a few seconds. Results from different laboratories performed on the same patient require that all the factors governing the test be recorded and then there must be someone with the knowledge to interpret them.

Quick's test is sensitive to factors I, II, V and VII. It should be noted that factor I (fibrinogen) and factor V (proaccelerin) are not affected by therapy, but they may influence the result of the test. It is particularly sensitive to factor VII (proconvertin); so much so in fact, that it is a reliable method of assaying this factor. This test, however, with its inherent weaknesses, served its purpose surprisingly well, mainly because as a general rule all the four factors involved, some of which were then unknown, came are depressed in parellel. As further facts came to light concerning the clotting mechanism, it became obvious that a more accurate test would have to be evolved. Other

factors were found which though affected by oral anti-coagulants, were not covered by the Quick method of control. If these factors were disproportionately decreased, haemorrhage was likely to occur; but of course the Quick result gave the patient's clotting time as safely in the therapeutic control range — not a very happy position.

In 1951 Professor Owren[5] of the University of Oslo produced a method which was sensitive to factor II (prothrombin) and factor VII (proconvertin) and which is known as the P & P test. This test which was designed to overcome some of the disadvantages of Quick's method is more sensitive to factor II and VII and also slightly sensitive to factor IX in as much as it will detect and register changes in this factor at approximately the point where haemorrhage is likely to occur. It is not sensitive to factors I and V which were two of the spanners in the Quick works. It is in fact a cleaned-up Quick's test.

This P & P method has two disadvantages which prevent it from being more widely used:—

1. The reagents can be made and stabilised only in a large laboratory, and this fact also carries with it the point that with the greater distribution of manufacture, the greater the likelihood of variation in the final product as more and more of the larger laboratories make their own.

2. It requires a skilled technologist to do this and the test itself. This of course means at least two skilled technologists for obvious reasons. You may think that skilled technologists are what is expected in any well-conducted laboratory, large or small; certainly it is desirable, but two trained especially to do one test, even though they will do other work as well most of the time, is rather a drain on manpower in a three or four-man laboratory. Furthermore, anticoagulant control will still be in demand even in a one-man set-up. Owren himself realised this and he also reported that with the more sensitive P & P test he still had bleeding episodes occurring (20,000 cases were tested and he controlled the treatment himself).

Statistics had by then established the fact that the patient who was held at an anticoagulant level not far short of the point of haemorrhage, had the best chances of survival[6]. For example, the Thrombotest level[4] at which it is stated haemorrhage will occur is 5% coagulation activity — the recommended therapeutic level is between 10% and 20% for both acute and ambulatory cases. To hold a patient safely at this level, a control test would have to measure accurately all four factors involved at a point inside the safety margin. It would have to be reliable and stable. It would have to be a test that if done in another laboratory anywhere, the results could be easily and correctly compared. It would be a help if it was relatively simple to perform and a stable

reagent was available commercially. Cost too, is important but it must be remembered that this is one of the few laboratory tests which has a direct and almost immediate effect on a patient's life—too much and he bleeds, too little and he has another and perhaps final thrombosis.

In November 1959 Owren[1] produced a test which measured up well to these requirements; he called it Thrombotest. Its sensitivity to factors II and VII was at least equal to P & P and it reacted to factor IX when its level was decreased below 10%. It has been shown that progressive prolongation of the secondary bleeding time occurs when factor IX levels are decreased below 10%.

Further, Thrombotest was sensitive to factor X (Stuart-Prower) which is also closely connected with bleeding episodes. Results have shown that as a general rule this factor is the most depressed by therapy and several accidental haemorrhages have been traced to it. Factor VII and prothrombin follow factor X in order of depression while factor IX remains at a higher level. It is the exceptions to this order which cause the trouble.

Opinions differ sharply as to whether in fact Thrombotest does show sensitivity to factor IX. Owen's experiments *in vitro*, which have since been confirmed by others, suggest that it does. The difficulty appears to be mainly in the *in vivo* material available. Sufferers of Christmas disease have a defect in the intrinsic system only. Because of the competition with the normal, faster extrinsic system in these patients, mainly factor VII, the decrease in factor IX is masked and shows up in the test as an increase in clotting time of only about 3 seconds. This of course, is inconclusive. It is necessary to have both systems depressed in parallel as in those on anticoagulants, and then to have a disproportionate decrease in factor IX, before conclusive results can be hoped for. Naturally one is loth to hold a patient at a dangerously low level just to see what happens, and bleeding episodes do not seem to occur if the level is above 10%.

One might say, "Why bother about factor IX?" To quote Owren[2]—"It may be correct to say that all clotting factors take part in the haemostatic mechanism, but under the circumstances of anticoagulant therapy, the most decisive mechanism for producing haemorrhage seems to be excessive reduction of factor IX. First, factor VII and factor IX are usually more depressed than the other factors." (This, of course, refers to those cases who are haemorrhaging and not to the usual order.) "Secondly, patients deficient in factor IX are more liable to bleed than those deficient in factor VII. Patients deficient in factor VII have no great bleeding tendency, and have normal secondary bleeding times.

For these reasons it is logical and theoretically sound to include factor IX, but the clinical importance of this determination remains to be further illustrated."

It should be emphasized that Thrombotest sensitivity to factor IX is detectable only at levels below 10%, but this is all that is required in the way of warning of impending haemorrhage. The closer the approach to 100% coagulation activity or normal, the less sensitive the test becomes to all factors. It was designed for patients on anticoagulant therapy and as such it performs an excellent job. And here a word of warning—because of the relative insensitivity at normal levels, it is impossible to establish a normal result from this test other than inside a very wide range —anything, in fact, from 80% to 120% may be normal. The 100% level of coagulation activity on any batch produced is established from a standard assayed normal reference plasma, not a random chance.

One of the main advantages[6] of the test is that because it evaluates all four factors, the result shows a trend, in that a decrease in percentage indicates a drift towards haemorrhage level. Further tests will show how fast this drift is and a slight correction of dosage carried out a week before the danger point is reached, will keep the patient at the optimum therapeutic level without the drastic changes necessary when using the one-stage test, which gives no indication of what the other factors are doing until they have done it. There will always be some cases which seem out of control, not including those which react unfavourably to the drug itself, such as those with liver damage. It has been stated[6] that in the big London clinics the greatest number of patients under full control at any given time was at best 80%. These patients were controlled by Thrombotest, and the cause of trouble in the case of 20% proved to be with the patients themselves, the test correctly showed their position. Reliability is one of its main virtues and troubles can safely be sought outside rather than in the test itself.

Dr Rosemary Biggs[3] in recommending its adoption said of it, that the danger of the test lay in the fact that anybody could do it. Simple as it is to do it should still be in the hands of at least a trainee, not just the office boy.

For those of you in country laboratories serving large areas, who face the difficulties of transporting specimens over long distances, I have here a study of 22 cases carried out by Mr Michael McCarthy of Laboratory Diagnostic Services, who has kindly made them available, giving Thrombotest results from tests done within one hour of venipuncture, 8-10 hours later and also 24 hours after taking off the blood. In the therapeutic range (10-20%), the greatest deviation in any one specimen was 1%

lower after 24 hours, with almost half showing no change in results. A similar picture was presented in the group below 10%, while those in the normal range, that is above the therapeutic range, showed as would be expected, much greater deviation—in a few cases up to 10%. As these were all in the 70-100% group, the difference is of no significance as they are virtually untreated. A further comparison was made using the diluted plasma technique as against whole blood. A deviation of up to 5% was found in one or two cases, but again these were above the upper limits of the therapeutic range, while those within the range were at worst 1%. In all cases the whole blood method gave the higher result. Thrombotest by the capillary technique showed only a slight variation. The blood in all cases of venipuncture, was collected by siliconised syringes into plastic containers.

Mr McCarthy also mentioned a case of interest, where the Thrombotest result was 11% and Quick's test was 26sec. At this level the patient had another coronary, and the attending doctor rather lost faith in the Thrombotest. A possible explanation for this rather rare occurrence, is that factor IX was not sufficiently depressed by the therapy, and therefore the intrinsic system was not under the influence of the anticoagulant. As thrombosis forms in the blood vessels due in the beginning to adhesion of platelets to the vessel walls, the intrinsic system is of vital importance. 11% Thrombotest would be a reflection of factor VII depression, as the influence of factor IX is not registered by the test much above 10%, and therefore its escape would not show in the result. It might be said that this fortunately rare picture is one of haemorrhage in reverse, with factor IX again playing the leading role. Possibly a level of 8% might be more suitable for this case, although very careful supervision would be necessary. Assays of the factors involved, carried out at the time of the episode would be needed to prove the point, but unfortunately this was not possible in this case.

Turning now to the constituents of Thrombotest[3]. The reagent is of the "all-in-one" type consisting of a slow acting thromboplastin and an accelerator in the form of cephalin. These two bring the extrinsic and intrinsic systems respectively into line, so that they will function at about the same speed. Added to this is a substrate plasma[10] having a high and constant content of the clotting factors not influenced by oral anticoagulants, but freed by absorption of the four factors to be evaluated, plus of course, calcium. It is extremely stable, having a shelf life of at least 12 months, and after reconstituting may be kept for some time in a deep frozen state. Further, there are only two places of manufacture; one under licence, so that variation in batches is kept to a minimum.

From the technical angle a few points are worth noting:—

1. Glass tubes and pipettes are used to do the test because at this stage, contact activation should be introduced.

2. We are all familiar with what might be called the "Quick flick." It is vital that the test is disturbed as little as possible after it has been started. Shaking and jolting will break up the small fibrin strands starting to form, and thus prolong the clotting time.

3. It is unnecessary to duplicate this test, as errors of up to 5sec made in reading the end point make almost no difference when converted to percentage coagulation activity.

4. One phenomenon which may be encountered is the appearance of what looks like agglutination. This should be taken as the end point, and usually occurs in patients whose level of anticoagulation is very near that of haemorrhage. It is in fact a collection of very small clots which will form into one solid clot if left untouched.

Provided the instructions are followed to the letter no difficulties with the test should be encountered. In our laboratory we have found that we can do the day's tests faster than by Quick's method.

And now a few words about the cost—well, the reagent costs 9d per test. This may appear high against the price of thromboplastin, but fewer tests are necessary, and there are no worries, no duplicating, and most important, no accidental haemorrhage and a much better control for the patient, who after all, is really the most concerned.

In passing, has anyone ever worked out the cost of the gallons of alcohol, stains, reagents and chemicals which are poured down the laboratory sinks of this country every day?

In summing up I would say this—Owren's P & P method should prove satisfactory in a large hospital capable of producing the reagents, and where the patients are controlled and are under the constant supervision of trained staff; but for those patients at home who have control over the drug they are taking, and who have little or no supervision, Thrombotest would seem to give a much safer coverage. Fortunately, the two tests are reported in the same way and correlate reasonably well, so that the change from hospital to the patient's home should follow without difficulty.

I hope I may be forgiven if I appear to have been selling Thrombotest, but after 10 years of worry over unstable thromboplastins and misnamed prothrombin times averaging 60 tests per day in duplicate, which leap up and down for no apparent reason, followed by the finger of fate in the form of the attending physician asking "why," when there is no ready answer, it is

indeed refreshing to find a test which appears to give a reliable result, and at the same time is free of all the uncertainties which existed before.

Undoubtedly modifications will be forthcoming as new discoveries are made in this field, but until the time comes when we can siliconise the walls of the blood vessels, anticoagulants and their attendant controls seem inevitable.

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Yeast, Glucose and Steroids

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Glucose interferes with several of the common methods of estimating steroids in urine, and yeast fermentation has been recommended as a way of removing it [4 5]. In the course of work on group methods for estimating the urinary metabolites of cortisol and cortisone (which are known as 17-hydroxycorticosteroids) we have found that yeast fermentation often produces grossly misleading results, and as this procedure for removing urinary glucose is in practise in some laboratories, it seemed advisable to write about it.

The presence of glucose cannot be tolerated in two main groups of steroid tests—those derived from the Reddy modification[8] of the Porter-Silber method[7] and those derived from the Norymberski method[1]. Glucose is insoluble in solvents such as chloroform and methylene dichloride, so any method which involves a preliminary extraction into these solvents is not affected by the presence of urinary glucose. This means that it does not interfere with the usual methods for estimating 17-ketosteroids, or for estimating free cortisol[2].

Cortisol and cortisone, like most other steroids, are excreted in the urine conjugated to glucuronide or to sulphate, and in general these conjugates must be hydrolysed prior to estimation. Only a small proportion of the total steroids produced by the body is excreted as the free, unconjugated compounds.

The Porter-Silber method measures corticosteroids which have a 17-dihydroxy-acetone group. It has been our custom to use the Reddy modification which involves extracting both the free and the conjugated steroids into butanol, and measuring the coloured complex formed with phenyl hydrazine at 410mμ. Unfortunately glucose, like other sugars, is freely soluble in butanol, and it also forms a compound with phenyl hydrazine which absorbs light at 410mμ. For this reason it is essential to remove glucose from urines before doing Porter-Silber estimations on them. In this laboratory yeast fermentation was tried for this purpose, and tested by comparing the apparent steroid levels in—

(1) a glucose free urine, and

(2) the same urine to which 2g/100ml glucose had been added, and then completely removed by fermentation with yeast. All traces of solid yeast were centrifuged out.

TABLE I

Porter-Silber steroid levels (mg/litre) in glucose free and fermented urines.

Urine	Glucose free	2% glucose removed with yeast
1	6.4	25.9
2	1.2	2.7

Each figure is the average of 3 or 4 estimations.

It is clear that yeast causes high and misleading results. Glucose does not interfere with the original procedure described by Porter and Silber for they, being mainly interested in the estimation of unconjugated steroids, used chloroform for extraction. When they wished to measure total 17-hydroxycorticosteroids in urine they hydrolysed the conjugated steroids before extracting them into chloroform. Reddy introduced the use of butanol as a solvent because, as both free and conjugated steroids dissolve in it, the need for preliminary steroid hydrolysis was eliminated. This at first seemed a considerable advance because the known methods of hydrolysing steroids are notoriously unreliable, the acid procedures being destructive, and the enzyme procedures variable. Unfortunately, however, later workers[9] have found that butanol also extracts from urine many substances which interfere with the Porter-Silber estimation. Sugars, ascorbic acid, urinary aldehydes and ketones, iodides, quinine, and even the dietetic breakdown products of spinach are among the many compounds which form coloured complexes with phenyl hydrazine and absorb light at 410mμ. New modifications [10] have recently been described which make it possible reliably to estimate 17-hydroxycorticosteroids in unhydrolysed urines by the Porter-Silber method.

In the Norymberski method the 17-hydroxycorticosteroids are converted to 17-ketosteroids and then estimated by the Zimmerman colour reaction with m-dinitrobenzene. This enables 17-hydroxycorticosteroids having either a hydroxyl (—OH) or a keto (=O) group at position 20 on the steroid molecule to be measured. We use a modification of this method[3, 6] in which the 17-hydroxycorticosteroids are changed to 17-ketosteroids by reduction with sodium borohydride followed by oxidation with sodium metaperiodate. Both these reagents are destroyed by glucose, each molecule removing from solution one molecule of borohydride and five of metaperiodate. After this the ketosteroids are extracted into chloroform in which glucose, being insoluble, cannot interfere.

The effect of yeast fermentation on urines analysed by the Few method[3] was tested as described earlier in the section on the Porter-Silber results.

TABLE II

Few steroid levels (mg/litre) in glucose free and fermented urines.

Urine	Glucose free	2% glucose removed with yeast
1	29.0	12.5
2	6.7	5.7
3	9.0	6.3
4	13.5	4.5

Each figure is the average of 4 estimations.

It can be seen here that yeast fermentation causes low results. Fortunately, however, glucose interference is no problem in the Few method because the addition of the appropriate extra amounts of sodium borohydride and of sodium metaperiodate reliably compensate for the presence of glucose[6]. Thus there is no need to remove glucose from urines which are to be analysed by this method.

TABLE III

Few steroid levels (mg/litre) in urines with and without 2% of added glucose.

Urine	Glucose free	2% glucose present
1	13.5	13.5
2	13.0	13.2

Each figure is the average of 4 estimations.

Summary

Yeast fermentation causes falsely low results in steroids estimated by methods derived from the Norymberski procedure, and falsely high results in steroids extracted into butanol and estimated by the Porter-Silber procedure.

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TECHNICAL NOTES

A Sterile 'Closed-Circuit' Method For Preparation of Red Cell Concentrates

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(Received for publication January 1963.)

A number of methods of preparing red cell concentrates are in use, ranging from pouring-off of the supernatent plasma, to aspiration of the red cells or plasma by means of a vacuum pump. In most cases the aspirated plasma is rendered unsterile and has to be discarded. The method described below uses a completely sterile 'closed-circuit' and the plasma may thus be used for inclusion in 'pooled plasma' for the manufacture of Human Albumin, etc.

Apparatus Needed

(1) M.R.C. or Abbot ½-litre bottles, containing a high vacuum.

(2) Packing Sets comprising:
1 x 7in needle of gauge 12 stainless steel tubing.
1 x 1in-1½in of gauge 15 stainless steel tubing.
1 airway needle (as supplied with donor packs).
15in rubber tubing 3/10in internal diameter.
Needle guards.

Method of Preparing Apparatus

(1) High-Vacuum Bottles.

½ litre M.R.C. or Abbot bottles are autoclaved so as to produce a high vacuum. This can be done by autoclaving at 15lb pressure for 20min. with the rubber stoppers of the bottles loose. At completion of autoclaving either allow to cool to 100°C then open the autoclave immediately and press rubber stopper in whilst bottles are still hot; or, whilst the autoclave is still at 15lb, blow the steam out and open the autoclave and quickly stopper bottles. These techniques give reasonable vacuums as long as no more than 10-12 bottles are sterilised in one batch. A much more satisfactory vacuum can be obtained with the use of a special apparatus for creating high vacuum in infusion bottles (see footnote). Bottles sterilised by this method should keep their vacuum for 3 months. It is essential to fit new, unperforated rubber bungs when resterilising, as a small puncture will allow leakage of air into the bottles.

Footnote: Obtainable from H. T. Frost (Instrument Maker) P.O. Box 499, Auckland.

(2) Packing Sets.

The long 7in needle is fitted into one end of the 15in rubber tubing, and the 1in needle into the other. Needles from used donor packs are suitable for this short needle. These needles, and the airway needle are guarded with either rubber or glass tubing.

Two complete packs, individually wrapped, are placed in suitable containers for sterilisation by autoclaving ('Gypsona' 5in x 15in tins with holes drilled in the ends to allow passage of steam can be used) and wrapped in linen and autoclaved.

Method of Packing Cells:

Fresh units of blood, which have settled and show a clear line of demarcation between plasma and cells are Cross-Matched by recommended methods. Compatible blood should be kept undisturbed and packed immediately prior to use. As this method of preparation is completely sterile, two units may be packed at one time.

The covers are removed from the blood bottles and the caps swabbed with antiseptic solution. The airway needle is introduced into one quadrant of each stopper and then the 7in needle introduced into the opposite quadrant and pushed in until the point is just above the plasma/cell interface.

Then the short needles on the other end of the rubber tubing are simultaneously pushed into the vacuum bottle. The plasma will then be aspirated from both units of blood. Should the plasma from one bottle be aspirated before the other, the short needle of this set should be immediately removed from the vacuum bottle. This will stop air being sucked through the transfusion unit and also stop any vacuum from being wasted, the other unit will continue to pack.

The aspirated plasma obtained in this manner is sterile, and can be used with expired transfusion units for pooled plasma for preparation of Human Albumin, etc.

Summary:

A sterile 'closed-circuit' method of preparing red cell concentrates is described. By this method the blood is less liable to contamination than by many other methods. Also the aspirated plasma remains sterile and can be used instead of being discarded as usual. The apparatus used is easily obtainable, inexpensive and suitable for smaller hospitals.

Abstracts From Other Journals.

Contributors to this issue: R. D. Allan, J. Case, J. Davies, J. Rees and H. C. W. Shott.

BLOOD BANKING

The Preservation of Human Red Blood Cell Agglutinogens in Liquid Nitrogen. Brousan, W. R. and McGinniss, M. H. (1962), *Blood* 20,478.

A simple method for the preservation of erythrocytes in liquid nitrogen suitable for routine laboratory use is described. The erythrocyte antigens retain their integrity for a least six months, and after thawing, remain active for at least two weeks in Alsever's Solution. The advantages of this technique over other methods of red cell preservation is discussed. J.R.

Blood Transfusion Hazards. Zeitlin, R. (1962), *Med. Sci. & L.*, 2,294.

The author discusses the technical and non-technical hazards of blood transfusion, and gives examples of human error as a factor in transfusion mishaps. J.C.

A Simple and Practical Method for Concentrating Blood Group Antibodies. Giblett, Eloise R. and Brooks, Lucy E. (1962), *Transfusion*, 2,261.

Serum is dialysed against pulverised dry Carbowax 20-M, a glycol polymer with a molecular weight of 20,000. The serum proteins including blood group antibodies and complement components are rapidly and economically concentrated. The value of this technique for identifying weak antibodies, converting rare but unreliable antisera into useful reagents, and the preparation of sera with high complement activity is discussed. J.C.

A Method of Producing Potent Anti-Non-Gamma Globulin in the Rabbit. Gold, E.M. and Lockyer, J. (1961), *Vox Sang.*, 6,615.

A1 cells are treated with formal saline and then sensitised with immune type Anti-A from a group O donor. The antigen - antibody complex binds complement, and this on injection into rabbits, produces anti-non-gamma globulin. The method has the advantage that no previous immunisation of the rabbit with a soluble antigen, and no adsorption of complement by the antigen-antibody complex, is necessary. J.R.

Delayed Haemolytic Reaction Caused by Anti-c Not Detectable, Before Transfusion. Roy R. B. and Lotto, W. N. (1962), *Transfusion*, 2,342.

A patient received ten units of apparently compatible blood during an orthopaedic operation. Five days later a moderately severe haemolytic reaction developed. A recheck of his pretransfusion serum revealed no antibodies but a sample taken on the ninth post-operative day contained a high titre Anti-c. J. C.

Blood Donors with a Positive Serological Test for Syphilis After Recent Successful Smallpox Vaccination. Mathieson, D. R. (1962), *Transfusion*, 2, 341.

Attention is again drawn to the prevalence of false positive flocculation tests following successful smallpox vaccination. The author discusses the problem created in blood banks in the event of a widespread vaccination programme, and warns against ascribing positive results to syphilis. J. C.

CHEMICAL PATHOLOGY

Chemical and Diagnostic Specificity of Laboratory Tests. Caraway, W. T. (1962), *Amer. J. clin. Path.*, 37, 445-464.

This paper deals with a survey and discussion of variables and potential sources of error in the individual specimen that affect the chemical specificity of clinical laboratory tests. Special attention is directed to factors less subject to correction by means of the usual techniques of quality control.

These include considerations of chemical specificity, anticoagulants, stability of specimens, haemolysis, lipaemia, control of temperature and pH, contaminations, effect of medications and normal physiological variations in persons.

A number of so-called pathognomonic tests have also been examined with respect to their diagnostic specificity. These include (1) biochemical tests for cancer and pregnancy; (2) the p-toluenesulphonic acid test for lupus erythematosis; (3) leucine amino-peptidase for carcinoma of the pancreas; (4) amylase for acute pancreatitis; (5) acid phosphatase for carcinoma of the prostate gland; (6) porphobilinogen for acute porphyria; (7) Congo-red absorption for amyloidosis and (8) Bence-Jones protein for multiple myeloma. (Author's own summary with anglicised spelling.) J. D.

Recent References to the Zimmermann Reaction: R. D. A.

A Rapid Routine Method for 17-ketosteroids. Green, A. G. (1962), *Clin. chim. Acta.*, **7**, 674.

The method is essentially that of Callow using an ether extraction to expedite the final drying. Colour development is carried out in the refrigerator at 4°C. for 60 minutes. This improves the colour, reducing the brown tints in urine extracts and results in lower blanks. Readings are made at 520 mμ only. A linear calibration curve from 0-80 μg. dehydroisoandrosterone is obtained. (Preliminary trials in our laboratory gave blanks of Ext. 0.020 and improved colour. Shorter development times were unsatisfactory. Low temperature colour development was described by Friedman as summarised below.

Spectrophotometric Studies of the Zimmermann Reaction. Friedman, H. S. (1959), *Clin. Chem.*, **5**, 575.

The effect of time, temperature and reagent concentrations was studied. About 90% of colour develops after 30 minutes at optimum reagent concentrations. Blank colour develops in alcoholic alkali above 0°C. but not in aqueous alkali: however, the alcoholic alkali gives a stronger reaction and 2.5N NaOH in 80% ethanol is used as a compromise. The steroid extracts are treated for 60 minutes at 0°C. with 0.4 ml. dinitrobenzene. Direct readings at 520mμ interpolated in standard graph. If necessary test blanks treated in parallel but omitting dinitrobenzene, are subtracted from the tests.

Methodology of Urinary 17-ketosteroids. Peterson, R. E. and Pierce, C. E. from *Lipoids and the Steroid Hormones* (1960), ed. Sunderman, F. V. and Sunderman, F. W., p.159, J. B. Lippincott, Philadelphia.

Dichlormethane — Soluble Red Colour. This extracts ketosteroids with an absorption maximum at 520mμ. 3-, 11- and 20-ketosteroids give some colour with the Zimmerman reagents, but very much less than 17-ketosteroids, and their absorption maximum is not at 520 mμ. After normal colour development, add 3 ml. 50% ethanol then 3 ml. dichlormethane. Stopper and extract gently for 10 seconds. (The authors say shake vigorously, but in our hands this results in obstinate emulsions and separation into phases). Allow to separate for 5 minutes in the dark and read at 520 mμ. It may be necessary to add a drop of ethanol to the dry cuvette to avoid cloudiness. (A dark coloured supernatant results from extraction and this we remove completely at the vacuum pump.)

HAEMATOLOGY

An Agglutination Test for the Differentiation of Leukaemoid States from Leukaemia. Slater, C. and Mitchell, M. A. (1962), *Amer. J. med. Technol*, **28**, 271-276.

A leucocyte agglutination test using guinea pig serum is described. By its use, myeloid leukaemoid reactions can be differentiated from acute and chronic granulocytic leukaemias. No conclusive information was obtained regarding the factors responsible for leucocyte agglutination. J. R.

A Two-stage Indirect L. E. Test. Lachman, P. J. (1962). *Immunology*, **4**, 142-152.

The technique and results of a two-stage indirect L. E. cell test are described. An account of the course of the lupus phenomenon as it occurs in this test, is given. Details are presented of a patient with systematic L. E., whose Direct reaction was persistently negative in the presence of a strongly positive Indirect reaction. (Author's own summary). J. R.

The Preparation of Stained Blood Films Using a Plastic Strip. MacKenzie, R. D. (1962), *J. med. Lab. Technol*, **19**, 184.

Polypropylene cast film obtained in inch-wide rolls presents a suitable surface for the preparation of blood films for haematological examination. Advantages are said to be the relatively low cost of the plastic strip, convenience under field conditions, low weight, and the lack of any need for elaborate packing for transportation. J. C.

A New Technique for Separation of Human Leucocytes. Lalezari, P. (1962), *Blood*, **19**, 109.

By differential sedimentation following red cell and platelet agglutination with Polybrene, over 50 per cent. of polymorphonuclears are recovered. Lymphocytes are partially removed. This technique is not applicable to rabbit leucocytes, but with other laboratory animals such as rats, mice or dogs, leucocyte separation can be readily achieved. J. R.

The Action of Serum on Plasma Thromboplastin. Nour-Eldin, F. (1962), *Acta haemat.*, **28**, 265-272.

When carrying out thromboplastin generation tests, it is well known that the serum dilution of 1 in 10 has to be made at least one hour before hand in order to obtain consistent results. This paper describes tests to explore the reasons for the undesirability of using freshly diluted serum. J. C.

Presence of a Human Blood Group Agglutinin in Thrombotest Reagent. Poller, L. and Barber, E. (1962), *Acta haemat.*, **28**, 326-328.

The presence of a non-specific blood group agglutinin in some batches of Thrombotest reagent is reported. When using the whole blood method with Thrombotest, this introduces a difficulty in reading the end-point, particularly in the longer clotting times. J. C.

MICROBIOLOGY

Methods of Testing Combined Antibiotic Bacteriocidal Action. Garrod, L. P. and Waterworth, Pamela N. (1962), *J. clin. Path.*, **15**, 328.

A description is given of two methods of measuring combined antibiotic bacteriocidal action; a test in liquid medium with subculture and particularly the cellophane transfer method. Technically the replica plating method is within the scope of the small hospital laboratory. It is emphasised that information so obtained is necessary in order to predict the effect of combined treatment, particularly in bacterial endocarditis due to organisms not fully sensitive to penicillin. The cellophane transfer method was applied to the study of the nature of combined antibiotic action on multiple strains of several species. The results were rarely uniform for any given combination and species; the necessity for individual tests as a guide to treatment is thus confirmed. H. C. W. S.

Antibacterial Activity of the Penicillins. Barber, M. and Waterworth, Pamela M. (1962), *Brit. med. J.* **i**, 1159.

With the advent of the new penicillins, the observations of Barber and Waterworth are both timely and helpful. Such a comparative study has not only given some valuable information regarding the action of these drugs, but has also sounded a note of caution regarding their indiscriminate use. H. C. W. S.

Listeria monocytogenes **and Listeria infection in the Diagnostic Laboratory.** *(1962). Ann. N.Y. Acad. Sci.*, 98, 686.

This review article covers the clinical significance and distinctive cultural characteristics of this group of organisms. Although the pathogenic role of these strains is sometimes in doubt, it is evident that the diagnosis of a Listeria infection is dependent upon the isolation of the organism rather than the serological evidence. H. C. W. S.

Genital Listeriosis as a Cause of Repeated Abortion. Rappaport, F., Rabinovitz, M., Krochik, N. and Toaff, R. (1960), *Lancet*, i, 1273.

Considerable evidence has been offered whereby a relationship between Listeria strains and repeated abortion has been suggested. Because there is a tendency to ignore the presence of "diphtheroid-like" organisms when investigating cultural plates, the reader is referred to this article. It is of particular interest that following this publication, greater attention has been given to the classification of this rather elusive group of bacteria.

H. C. W. S.

Book Review.

Ciba Foundation Colloquia on Endocrinology, Vol. 14. Immunoassay of Hormones. Ed. G. E. W. Wolstenholme and Margaret P. Cameron. J. & A. Churchill Ltd. London 1962. 56s in U.K.

This latest contribution to the Ciba Colloquia deals with a subject of extreme importance in the clinical laboratory. It is the hope of all chemical pathologists and clinical endocrinologists that the always tedious and often impractical bioassay of protein hormones will eventually be replaced by a method more amenable to the operations of the routine laboratory. The book under review details the explosive progress in the last decade of the method most likely to succeed—immunoassay.

Professor F. G. Young's opening remarks present a brief history of the expansion of immunology beyond the bounds of bacteriology, and its application to problems connected with the identification and assay of proteins, and particularly of protein hormones. The various sections of the book then deal with (1) fundamentals of immunochemistry as applied to hormones, (2) growth hormones, (3) insulin, (4) glucagon, (5) thyrotropin, (6) corticotropin, (7) gonadotropins, and (8) prolactin.

Each section includes a series of papers by experts active in the field, as well as vigorous discussion by participants in the symposium. The discussion makes clear, not only that work is progressing actively on all fronts, but that there is also much contradiction and confusion in the field, which is far from ready to replace bioassay.

The present status of the subject, insofar as it affects clinical chemistry, is that although immunoassays of a sort have been devised for all the hormones mentioned above, the only one which appears currently practicable on a routine level is that for chorionic gonadotropin (indeed, an American firm is marketing a useful pregnancy test based on inhibition of agglutination of latex particles). All the other assays are not only difficult research procedures, but the significance of their results is hotly disputed by experts.

The book is recommended for two classes of readers: those who are actively engaged in research in the immunoassay of hormones, who will wish to study the entire book, and particularly the discussions; and those who would like to skim the contents to get the flavour of contemporary thought and action in a field which will certainly become important in the clinical laboratory.

Ralph Spitzer.
Visiting Senior Lecturer in Chemical Pathology
University of Otago.

The Health Department Examinations (Spring, 1962)

INTERMEDIATE (17th and 31st October, 1962)

Written Paper I (Haematology, Blood Bank Technique and Bacteriology).

All questions to be answered. Time allowed; three hours.
(Explanatory Note: Questions 1 to 40 required that the candidates indicated their selection by placing a cross under the appropriate letter on the special sheet provided.)

(1) Heller and Paul's mixture contains (a) potassium and sodium oxalates (b) potassium and ammonium oxalates (c) sodium and ammonium oxalates (d) ammonium and calcium oxalates.

(2) The above mixture prevents clotting by (a) removing the calcium as insoluble calcium oxalate (b) interfering with thrombin formation (c) deionising calcium (d) chelating calcium.

(3) Collecting blood in potassium oxalate (a) increases the reading of the haematocrit examination (b) does not affect the reading of the haematocrit examination (c) causes slight haemolysis (d) reduces the reading of the haematocrit.

(4) Heparin prevents blood clotting by (a) deionising calcium (b) neutralising calcium (c) neutralising potassium (d) neutralising thrombin.

(5) What strength sodium citrate is normally used as an anticoagulant (a) 0.7% (b) 7.5% (c) 3.8% (d) 10%.

(6) EDTA is (a) a sulphate (b) a chloride (c) a chelate (d) a nitrate.

(7) What protein is NOT found in serum (a) albumin (b) globulin (c) fibrinogen.

(8) What is the normal size of a polymorph (a) 7.2μ (b) 12μ (c) 20μ (d) 0.05mm.

(9) A micron is (a) 0.1mm. (b) 0.01mm. (c) 0.001mm. (d) 0.0001mm.

(10) Before being stained in Giemsa stain a smear is (a) haemolysed in water (b) fixed by heating (c) fixed in methyl alcohol (d) fixed in alcohol-ether.

(11) Leishman stain is present in solution in the amount of (a) 0.15% (b) 0.5% (c) 1% (d) 5%.

(12) Leishman stain is dissolved in (a) methyl alcohol (b) ethyl alcohol (c) propyl alcohol (d) caprylic alcohol.

(13) Romanowsky stains are best diluted with water buffered to pH (a) 5.2 (b) 6.8 (c) 7.4 (d) 7.8.

(14) Romanowsky stains are a mixture of (a) carbol fuchsin and methylene blue (b) crystal violet and neutral red (c) haematoxylin and eosin (d) eosin and methylene blue.

(15) Blood stains are buffered with (a) Arneth and Cooke's buffer (b) Schilling's buffer (c) Sorensen's buffer (d) Grubler's buffer.

(16) An increased reticulocyte count is shown in (a) red cell regeneration (b) white cell maturation (c) iron treatment of anaemia (d) sudden blood loss.

(17) Normal red cells show (a) 0.2% (b) 0.7% (c) 1.5% (d) 2% reticulocytes.

(18) Stippled cells are increased in (a) chronic alcoholism (b) chronic lead poisoning (c) tuberculosis (d) worm infestation.

(19) Leucopenia means (a) an increase in leucocytes (b) a decrease in leucocytes (c) a decrease in monocytes (d) a decrease in platelets.

(20) Adsorption means (a) the fastening of antibodies onto a surface (b) the elution of antibodies from a surface (c) the recovery of antibodies from a surface (d) the identification of antibodies on a surface.

(21) An agglutinin is a substance produced in serum in response to (a) a specific antigen (b) a specific antibody (c) a hapten (d) a chromatophore.

(22) Chromatin stains most readily with (a) basic dyes (b) acidic dyes (c) neutral dyes (d) neutralised dyes.

(23) Erythropoiesis means (a) the formation of red cells (b) the formation of white cells (c) the formation of platelets (d) the formation of Howell-Jolly bodies.

(24) Haemolysis means (a) the breaking up of red cells (b) the breaking up of white cells (c) the breaking up of platelets (d) the neutralisation of haemolysin.

(25) Hypotonic means (a) chemically normal (b) N/10 (c) below the pH of blood (d) with low osmotic pressure.

(26) Idiopathic refers to (a) a transmitted disease (b) an inherited disease (c) a disease of spontaneous origin (d) a disease peculiar to a country.

(27) Leucopoiesis means (a) the formation of leucocytes (b) the formation of platelets (c) the formation of red cells (d) a diminution of white cells.

(28) Macrocytes are found often in (a) aplastic anaemia (b) pernicious anaemia (c) microcytic anaemia (d) haemolytic anaemia.

(29) Polychromasia is a tendency for certain erythrocytes to stain with (a) both basic and acidic dyes (b) basic dyes (c) acidic dyes (d) Evans blue.

(30) Proteolytic refers to (a) digestion of fat (b) digestion of glucose (c) digestion of protein (d) digestion of peptone.

(31) Spherocytes occur particularly in (a) pernicious anaemia (b) aplastic anaemia (c) sickle cell anaemia (d) acholuric jaundice.

(32) Stromatin is (a) the framework of a cell (b) the envelope of a cell (c) the nucleus of a cell (d) the nucleolus of a cell.

(33) Titre is (a) the highest (b) the lowest (c) the mean (d) the reciprocal of the lowest dilution which will cause agglutination of an antibody with its corresponding antigen.

(34) $\frac{\text{Packed cell volume}}{\text{Erythrocytes in millions per cumm.}} \times 10$ is the formula for (a) M.C.H. (b) M.C.H.C. (c) M.C.V. (d) C.I.

(35) The addition of N/10 hydrochloric acid to haemoglobin forms (a) haematin (b) oxyhaemoglobin (c) methaemoglobin (d) carboxyhaemoglobin.

(36) The size of the side of the smallest square in a Neubauer counting chamber is (a) 50μ (b) 1/5mm. (c) 1/10mm. (d) 20μ.

(37) The myeloblast is the origin of (a) granular cells (b) leucocytes (c) monocytes (d) platelets.

(38) Worm infestation causes an increase in (a) polymorphs (b) lymphocytes (c) platelets (d) eosinophils.

(39) Which is the wrong chemical in the following Hayem's solution (a) sodium chloride (b) sodium sulphate (c) mercuric chloride (d) acetic acid.

(40) Coagulation time is estimated by the method of (a) Lee and White (b) Hess (c) Duke (d) Ivy.

(41) What materials and equipment would you supply to a doctor who is about to investigate a suspected case of typhoid fever; and what tests would you perform on the specimens supplied to you?

(42) Describe how you would prepare and standardise two litres of broth for bacteriological use, beginning with raw materials. Assume that the final product will be dispensed in 250 ml bottles. What do you mean by the term pH?

(43) Describe your technique for providing blood for transfusion in the following cases:—
(a) Immediate transfusion without typing or cross-matching.
(b) Immediate transfusion, but six minutes allowed for laboratory tests.
(c) Urgent transfusion, 45 minutes allowed for laboratory tests.
(d) Non-urgent transfusion.

Written Paper II (Biochemistry and General).
All question to be answered. Time allowed; three hours.

(1) Using diagrams to illustrate your answer, describe the action of a still for the production of high grade distilled water.

(2) Describe the composition, porosity and use of all filters which you employ in the Pathology Laboratory.

(3) Describe in detail the estimation of T.N.P.N. in blood. Give the theory upon which this estimation is based and using an imaginary example, show how the result is obtained.

(4) Describe in detail how you would make a litre of physiological normal saline and check its accuracy by chemical means.

Practical Papers
All questions to be answered. Ten minutes allowed for each question.
Group A

(1) Identify as far as possible from the cultures etc. supplied, this gram-negative non-motile organism which has been isolated from faeces. Draw out your scheme for the isolation of intestinal pathogenic micro-organisms.

(2) Give the probable identification of the organisms in slides A, B and C.

(3) Identify the given organism. What are the criteria for the separation of organisms in this group?

(4) What is this piece of equipment and how is it used in the laboratory? What precautions should be taken in its use?

(5) Perform a colony count on the plate provided and estimate the original count per ml. supposing that the plate contains 1 ml. of a 1 in 100 dilution of the original material.

(6) Test the urine supplied for bile and acetone.

Group B

(7) Draw a diagram of a colorimeter showing the optical path of the light, and describe the working of the instrument. Supposing that you are estimating glucose; your standard is equivalent to 200mg/100 ml. and your "unknown" has been found to be 312mg/100ml. What will be the lengths of each solution under the plunger in the colorimeter?

(8) Note the important points to be observed in the derivation of M.C.H. and give an example showing how this is calculated.

(9) Comment on the abnormalities which you find in the three slides D, E and F provided. Make brief notes on your findings.

(10) Comment of the efficacy of the disposal of the organisms in the lysol bath provided. Give a list and brief comment on the means of sterilising discarded material from the Bacteriology Department.

(11) Prepare three throat swabs from the material provided. How would these be sterilised?

(12) Write notes on the three spots provided.

Group C

(13) Estimate the amount of glucose in the urine provided.

(14) Examine the two urine deposits provided and comment on the abnormalities, if any.

(15) Describe the use of the burette supplied and the method you would adopt to check the accuracy of the markings.

(16) Titrate the specimen provided for free and total acidity. To what do the figures for acidity refer? Describe the mode of action of the indicators.

(17) Spread three films from the blood supplied and stain with Leishman stain. Give the differential features on the cells normally seen in a stained blood film.

(18) What are the components of the following solutions and what purpose does each serve in the solution?

(a) Platelet counting fluid.
(b) Erlich's Reagent.
(c) Esbach's Reagent.

Successful Candidates

Bond, Miss M. H. J. ... Wellington
Cullen, Miss H. ..... Napier
Culy, Miss P. ..... Wellington
Forsyth, A. ..... Dunedin
Lowry, C. F. ..... Christchurch
MacGibbon, Miss J. M. ... C'church
McRae, Miss K. M. ..... Gisborne
Mitchell, M. A. ..... Rotorua
Scott, Miss W. M. ..... Greymouth
Stark, Miss G. M. ..... Timaru
Turner, Miss P. A. ..... Whakatane

FINAL—CERTIFICATE OF PROFICIENCY

(15th, 16th, 29th and 30th August, 1962)

Written Paper I (Bacteriology).
All questions to be answered. Time allowed; three hours.

(1) A patient who has clinical symptoms of typhoid fever is admitted to your hospital. Give an account, under the following headings, of the bacteriological investigations which the laboratory could carry out. (a) The specimens from the patient which should be provided for examination *(5 marks)*. (b) Methods of culture and identification of the organism. State reasons for the use of special media *(15 marks)*. (c) Serological investigation *(5 marks)*. (d) Outline briefly how the laboratory could help in tracing the source of the infection *(5 marks)*.

(2) Describe the method you would use to isolate betahaemolytic streptococci from a throat swab *(10 marks)*. Outline the method used for *grouping* and *typing* betahaemolytic streptococci *(15 marks)*. What purpose is served by grouping and typing these organisms? *(5 marks)*.

(3) Describe how you would proceed to isolate *Candida albicans* from a specimen. State how you could distinguish this organism from other non-pathogenic Candida species *(15 marks)*. From what human sources is *Candida albicans* likely to be isolated as a pathogen? *(5 marks)*.

(4) Write brief notes on:—
(a) Exotoxin. (b) Endotoxin. (c) Haemagglutination. (d) Tuberculin. *(5 marks each)*.

Written Paper II (Biochemistry).
All questions to be answered. Time allowed three hours.

(1) Describe *three* different enzyme estimations which are carried out in the hospital biochemistry laboratory *(25 marks)*

(2) Give an approximate table showing the relative part played by the various constituents in maintaining the acid-base balance in the body fluids. What are the main factors which tend to upset the acid-base balance? Write brief notes on the technique of estimation of those electrolytes usually requested *(30 marks)*

(3) Discuss briefly the methods of estimating protein concentration in body fluids (including fractionation) *(25 marks)*

(4) Write short notes on any *five* of the following:—
(a) C.S.F. Sugar. (b) Sodium tungstate. (c) Optical density. (d) Hand spectroscope. (e) Precautions to be taken in the use of centrifuges. (f) Precautions to be taken in the handling of concentrated acids. (g) The use of bromine in the hospital biochemical laboratory and the precautions to be observed in its use. (h) Turbidimetric estimations *(4 marks each)*

Written Paper III (Haematology and Blood Bank Technique).
All questions to be answered. Time allowed three hours.

(1) Give an outline of the structure and functions of haemoglobin. Explain what is meant by adult and foetal haemoglobin, and describe one method of estimating the percentage of foetal haemoglobin present in a specimen of blood *(30 marks)*

(2) Write a short note (not more than half a page) on each of the following:—
(a) Autohaemolysis. (b) "Burr" cells. (c) Sex chromatin in leucocytes. (d) Evans Blue. (e) Ashby count *(20 marks)*

(3) Explain the difference between a complete and an incomplete antibody. Discuss the relative merits of the various methods which can be used for the detection and titration of incomplete antibodies *(25 marks)*

(4) What is the purpose of the haematological "screening" test? In what parts of the world would one be most likely to encounter the following conditions, and what might be seen in a blood film in each case? (a) Sickle cell anaemia. (b) Thalassaemia. (c) Filariasis *(25 marks)*

Practical Paper (Bacteriology).
Time 3 hours. *(Total marks 35)*

(1) Complement Titration

You are provided with reconstituted lyophilised guinea pig complement diluted to a strength of 1 in 10, Wassermann antigen, pooled W.R. human serum, sensitised sheep red cells, saline, 1ml graduated pipettes and a calibrated capillary pipette delivering drops of 0.02ml volume.

Titrate this complement to determine its minimum haemolytic dose for use in the Wassermann test.

Proceed as follows:

(a) Prepare saline dilutions of complement 1 in 10, 1 in 20 through to 1 in 70. State your method. *(8 marks)*

(b) Titrate the complement as follows:
Row 1 (7 tubes) to which are added
1 volume complement dilutions (1/10 to 1/70)
1 volume saline
One fifth volume of pooled normal (W.R. negative) serum
1 volume of Wassermann antigen.
Row 2 (7 tubes) to which are added
1 volume of complement dilutions (1/10 to 1/70)
2 volumes of saline
One fifth volume of normal serum
(Note: *Use 0.2ml as the unit volume. To measure the one fifth volume, use calibrated pipette).*
When the reagents have been added, incubate in 37°C water bath for 1 hour. Then add 1 volume of sensitised cells, return to water bath for ½ hour, read and record results. *(18 marks)*

Write briefly on the following:

(a) Why is complement titrated in the presence of normal serum, and antigen in the presence of normal serum? *(3 marks)*.

(b) What is used to sensitise the sheep red cells? *(3 marks)*.

(c) How are calibrated capillary pipettes prepared? *(3 marks)*.

(2) Examination of a mixed culture and preparation of a Nagler plate *(Total marks 40)*

The culture A provided was inoculated with material from a deep ulcer.

Proceed as follows:

(a) State what medium has been used and its particular advantages. *(5 marks)*

(b) Prepare a direct smear from the culture A and give a preliminary report on the organisms present. *(5 marks)*

(c) Inoculate a blood agar and a MacConkey's agar plate with material from culture A. These plates will be incubated overnight aero-

bically at 37°C and should be reported on tomorrow *(5 marks)*

(d) Prepare and inoculate a Nagler plate with culture A in such a way that *Cl. welchii*, if present may be identified. This culture will be incubated anaerobically at 37°C overnight and should be reported on tomorrow. The Nagler plate should contain 10% egg yolk and 100μg of neomycin per ml. State the methods you use. *(16 marks)*

You are provided with:

1. A petri dish containing a basal layer of nutrient agar.
2. 18ml of Nagler base melted and kept at 56°C.
3. Egg yolk.
4. Aqueous neomycin 10,000μgm/ml.
5. Diagnostic serum *Cl. welchii* type A.

Write brief comments on the following:

(a) The Nagler reaction. *(6 marks)*

(b) The use of neomycin in the medium. *(3 marks)*.

(3) Report on the following slides: *(Total marks 25)*

Slide A—A smear of pus from a suppurating lesion in the lower jaw of a 46-year-old male. Stain by Gram's method and state the probable identity of this organism. How would you attempt to culture it? *(6 marks)*
Actinomyces

Slide B—A smear from the centrifuged deposit of a cerebro-spinal fluid sample from a child aged 9 months. Stain by Gram's method and identify the organism. How would you culture it? *(4 marks)*
H. influenzae.

Slide C—A smear from ulcers in the mouth of an adult female patient. Stain by Gram's method and make a diagnosis. *(4 marks)*
Vincent's Angina

Slide D—A smear prepared from a pure culture. Stain by Gram's method, taking care not to over-decolourise. Describe the characters of this organism as seen in your preparation and state what organism it is. How would you separate this organism from a mixed culture? *(6 marks)*
Cl. tetani

Slide E—A slide culture of an organism obtained from the sputum of a patient with lung disease. What is this organism? Could it be the cause of the patient's disease? *(5 marks)*
Aspergillus fumigatus

Practical Paper (Biochemistry). *Time 3 hours*

1. Estimate the alkaline phosphate content of serum A.
2. Carry out an estimation of urinary diastase in specimen B.
3. Estimate the chloride content of serum C.
4. Report on the spot tests labelled 1 to 5.
5. Estimate the bilirubin content of serum D.
6. Report on the spot tests labelled 6 to 10.

Practical Paper (Haematology and Blood Bank Technique). *Time 3 hours*

1. You are provided with three sera labelled A, B and C. Using the Coombs' technique and the washed, packed group O D-positive cells supplied, test each serum for antibody. Determine the titre of any antibody found.

2. Examine the five blood films labelled 1 to 5 inclusive. Describe any abnormalities and suggest the appropriate diagnosis if possible. Do a differential leucocyte count on film No. 5.

3. Write a short note on each of the "spots" numbered 1 to 10.

Successful Candidates

Brown, T. E. ..... Dunedin	Mitchell, D. F. ..... Whangarei
Hains, G. D. ..... Hamilton	Ranford, Miss H. M. Wellington
Mackintosh, Miss J. E. Wellington	Wolley, Miss J. A. ..... Wellington

Nine candidates sat this examination; one withdrew during the examination and two gained a partial pass. The average pass mark was 59%.

Branch Reports.

AUCKLAND

(Secretary: G. Donn de Silva, Pathology Department, Public Hospital, Auckland).

During 1962, members of the Branch participated in various functions as follows:

1. *Monthly meetings* addressed by notable speakers including:—
   Dr P. Brooke "*Facial Eczema*"
   Dr N. Dalton "*Life in Nigeria*"
   Dr J. Sinclair "*Medical Research*"
   Dr B. Cain "*Cancer Research*"
   Dr W. M. Manchester "*The Scope of Plastic Surgery*"
2. *Conducted tours* of the new laboratories and plant of Biological Laboratories Ltd., and Cooper, McDougall and Robertson Ltd.
3. *One-day Conference.* The Branch organised a very successful one-day Conference in November at which technologists from as far afield as Whangarei and Taumaranui heard papers presented on many subjects of interest. The programme concluded with a buffet tea and social evening.

DUNEDIN

(Secretary: E. K. Fletcher, Pathology Department, Medical School, Dunedin.)

The final meeting of 1962 was held at Kew Hospital, Invercargill, an excellent showing of members travelling from Dunedin. Two papers were presented during the evening:

"*Megaloblastic Anaemias*" by Dr T. Henshall and

"*The Identification of Blood Group Antibodies*" by Mr J. Case.

Following the meeting, Mr and Mrs G. C. Thompson acted as hosts at a social gathering in their home.

Events characteristic of the occasion ended 1962. A barbecue was held on December 8 at Outram Glen, with all present obviously having an enjoyable evening. Popular opinion seemed to favour this type of gathering rather than the more formal function originally planned.

The Branch looks forward to an active year commencing with the first meeting on March 7, at which it is hoped to welcome several new members. Prof. E. F. D'Ath, the retiring Professor of Pathology at the University of Otago, has been invited to address the meeting.

Continued close association with the Branch's more distant members, particularly in Invercargill, is hoped for; and with Dunedin as the venue for the 19th Annual Conference, the opportunity of being host to the many visitors in August, is welcomed.

WELLINGTON

(Secretary: G. Tait, c/o Drs Lynch, O'Brien and Desmond, 40 Riddiford Street, Wellington.)

Formerly, the Wellington Branch has utilised the talents of its members in the provision of lectures and demonstrations. This year it was decided to seek further afield, and to include topics not necessarily directly connected with medical laboratory technology. The programme for 1962 included talks from members of the medical profession with the object of providing a background of knowledge which can be related to technical laboratory procedures. This policy is of particular value to the junior members and has proved successful.

At the next meeting, some of the matters likely to be introduced at the Annual Conference will be discussed. It is hoped that by providing members with a clear picture of the issues involved, time may eventually be saved at the actual Conference.

Lectures to Date:

1. *"A Cytological World Tour,"* Mrs R. Parker, Wellington Hospital. Mrs Parker attended a conference on Cytology in Vienna, and then proceeded to the United States to study American techniques in this field.
2. *"Cervical Cancer,"* Dr P. Cairney, Wellington Hospital. Dr Cairney outlined the medical background of cervical cancer.
3. *"Some Aspects of Nuclear Physics,"* Dr N. McLoughlin, Nuclear Science Division. This lecture was given at the Hutt Hospital with the active co-operation of Mr Clarkson. Dr McLoughlin spoke and demonstrated most clearly the effects of different atomic particles, and applied them to some medical problems.
4. *"The Pathology of Coronary Thrombosis,"* Dr F. B. Desmond.

All lectures were preceded by a business meeting, and it pleasing to note that junior members are beginning to take an active part in the discussions.

Social Occasions:

By far the most successful meeting of the year was the Christmas cocktail party. This was the second since the inception of the Branch, and is likely to be a regular feature of each year's activities. The Branch had planned a trip to Blenheim at the invitation of Mr H. G. Bloore; unfortunately it proved impossible on this occasion to fit this in with the schedule of the *"Aramoana,"* but the idea has not been abandoned.

Council Notes.

A meeting of Council took place at Wellington Hospital on Saturday, 15th December, 1962. Those present were H. T. G. Olive (in the Chair), Miss J. Mattingley and Messrs H. Bloore, J. Case, M. Donnell, G. George, H. Hutchings, J. Morgan and D. Philip. An apology was received from Mrs J. Hodgetts (nee O'Grady) who was still away on her honeymoon.

Examinations

Ministerial approval has finally been given to the establishment of an Examination Board, which is to consist of three representatives of the New Zealand Society of Pathologists and four from the New Zealand Institute of Medical Laboratory Technology. A full-time secretary is to be employed, who will establish and maintain a register of trainees.

Commencing in 1963, both Final and Intermediate examinations will be conducted only once yearly. An entrance fee of £5 5s 0d and £3 3s 0d respectively will be payable by entrants, and there is to be provision for candidates who fail in any one subject in the Final examination to sit a special examination in that subject later in the year. The entrance fee for the special examination will be £2 2s 0d. Candidates will continue to sit the theoretical papers in their own laboratories, and the practical and oral sections of the examinations will be held approximately two weeks later at predetermined centres.

Examination syllabuses are still under consideration, and various suggestions are to be circulated to Council members for their comments, to be forwarded to the President before 28th February. It was generally agreed that the new syllabuses when published, should include sections applicable to the Intermediate, Certificate of Proficiency and Fellowship Examinations respectively.

The Journal

Council gave consideration to the new Journal Committee's plans for the Journal and to increase its circulation overseas. A proposal to change the name of the Journal was unanimously agreed, and the sum of £150 was voted for the establishment of a banking account in Dunedin. This sum was intended to cover expenses for 1963 and would be subject to review after the first year. The Journal Committee will in future conduct its own finances and will submit an audited balance sheet at the end of each financial year.

New Members

The decision taken at the 1962 Conference to circularise potential new members, will be implemented as soon as possible. Drafts of the proposed circular letter and the application form for membership were considered and approved. Recruitment is likely to be considerably accelerated by this method. Meanwhile, thirteen applications for junior membership were considered and approved:

August, Miss P. ..... Greymouth
Beal, Miss J. G. ..... Hamilton
Coulton Miss D. ..... Greymouth
Flack ..... Hastings
Gerring, Miss M. D. ..... Hamilton
Grant, Miss K. M. ..... Hamilton
Knight, Miss S. A. ..... Hamilton
Marsh, Miss R. ..... Lower Hutt
Paterson Miss E. M. F. ... Hamilton
Prior, Miss J. ..... Whakatane
Scott, Miss W. ..... Greymouth
Tibbles, B. ..... Greymouth
Turner, Miss P. A. ..... Whakatane

Resignations

Two resignations were accepted:
Ford, Miss A. ..... Invercargill
Hawke, V. J. ..... Nelson

New Rules

The time seems opportune for the revision of the Institute's Rules. The draft of the proposed new rules will be circularised to all members in time for consideration and approval at the 1963 Conference.

Dunedin Conference

Council gave its enthusiastic approval to Mr Allan's proposal to run the Conference on the lines of several seminars on various subjects. The 1963 Conference promises to be refreshingly different from those of former years, and members proposing to attend should find their attendance well worthwhile.

Finance

The credit balance stands at £439 6s 5d.

Hospital Employment Regulations.

The following letter, dated 24th January, 1963, has been received from the Department of Health by the Secretary:—

I am writing to inform you that just before Christmas the Department received approval from Government for improved salary scales and conditions of employment for some of the occupational groups covered by the Hospital Employment Regulations, to apply from April 1, 1962. These decisions resulted from the recommendations made by the appropriate Salary Advisory Committees last year and represent the extent to which submissions made by recognised Associations have been accepted.

Hospital Boards were given preliminary advice by circular on December 21 and are now being informed in detail and authorised to implement the approvals. This letter and the attached schedule will serve to inform recognised Associations and members of Advisory Committees of the approvals granted by Government which are the direct concern of the organisation or person in each case.

A. E. Galletly
for Director, Division of Hospitals.

The appropriate sections of the schedule are reproduced below.

C. MEDICAL TECHNOLOGISTS (BACTERIOLOGISTS).

		£
1.	Scale (a)	
		1,430
		1,530
		1,630
		1,730
2.	Regulation 5. Special maximum for a grade laboratory officer of exceptional qualifications and training, or of exceptional experience	1,930

D. HOSPITAL SCIENTIFIC OFFICERS
E. PHYSICISTS

1.	To commence at Public Service commencing rate for appointees with University Degrees, and advance on the following salary scale, with provision for double increments on the recommendation of a Board and the approval of the Minister of Health	835
		880
		930
		970
		1,015
		1,060
		1,105
		1,150
2.	Then to be promoted to the following scale on the recommendation of a Board and the approval of the Minister of Health	1,195
		1,240
		1,330

3. Senior Hospital Scientific Officers
Senior Physicists

The salaries of Senior Hospital Scientific Officers to be determined by the Hospital Medical Technologists Salaries Grading Committee and those of Senior Physicists by The Minister of Health; in both cases maximum to be determined within the following salary scale

1,430
1,530
1,630
1,730
1,830
1,930
2,045

G. MISCELLANEOUS PROVISIONS FOR LABORATORY WORKERS

1. Definitions in the Hospital Employment (Laboratory Workers) Regulations to be amended as follows:—

(a) The definitions of "Biochemist" and "Grade Laboratory Officer" are repealed and the term "Hospital Bacteriologist" is replaced by the term "Medical Laboratory Technologist."

(b) The following new or amended definitions are provided:—

"Grade Laboratory Officer" means a principal or sole medical laboratory technologist in any hospital or a senior hospital scientific officer, a tutor medical laboratory technologist and includes any other medical laboratory technologist who by reason of his special duties or responsibilities is for the purpose of these regulations designated by the Grading Committee as a grade laboratory officer.

"Laboratory Worker" includes a hospital scientific officer, a medical laboratory technologist, a trainee and a laboratory assistant.

"Hospital Scientific Officer" means a University graduate with an appropriate degree employed in a medical laboratory.

"Senior Hospital Scientific Officer" means a hospital scientific officer who is for the purpose of these regulations designated by the Grading Committee with the approval of the Minister as a senior hospital scientific officer.

"Tutor Medical Laboratory Technologist" means a medical laboratory technologist who is responsible for the teaching of trainees in a training school recognised by the Director-General and is wholly or mainly employed in that work.

2. Amendment of the overtime provisions of the Laboratory Workers Regulations to provide for Hospital Scientific Officers and Senior Hospital Scientific Officers on the same basis as other grade laboratory officers.

3. Laboratory Assistant. There is provision for the Grading Committee, with the approval of the Minister to determine a special rate of salary after the officer has been at least one year on the tenth year rate of the normal scale and the requirement for one year's service on the tenth year rate is to be deleted. Provision is also to be made within the normal scale for accelerated promotion with the approval of the Director-General.

Preliminary Notice

ANNUAL CONFERENCE 1963

The 1963 N.Z.I.M.L.T. Conference will be held at the Dunedin Medical School on Thursday and Friday, August 22 and 23. The dates have largely been determined by the University Calendar, but will provide extra time for the preparation of papers and demonstrations. The Council have agreed that we try a series of Symposia under four main headings, namely Chemical Pathology, Haematology, Microbiology and Education in relation to Medical Laboratory Technology.

The idea is to provide an opportunity to discuss these subjects with reasonable informality and freedom under the direction of a suitably qualified chairman. A programme of current topics is envisaged with introductions in the form of short papers.

Although each section will be presented consecutively, alternatives will be provided for those not wishing to attend a particular section. This may also provide an opportunity to study the trade exhibits with more deliberation than is usually possible.

In order to arrange the details of these programmes *I would be grateful for the following information before the end of May:*

1. Topics you would like to hear discussed.
2. Topics you are willing to contribute to and to what extent, thus—
   (a) Papers describing new techniques, surveys, systematic approach to specific problems or detailed descriptions, in short, the conventional paper requiring more than 10 minutes to read.
   (b) Brief introductions to specific problems or to a particular technique or modification.

It is hoped that many more people will be encouraged to discuss topics related to the daily grind or to speak on points which do not merit the full treatment.

In the first case the title of the paper and a brief indication of its scope would be appreciated. In the second case a concise resume is required so that the question can be discussed with or without further elaboration or by proxy.

In either case indicate if slides are to be shown.

3. Demonstrations and exhibits—
   (a) Nature of demonstration or exhibit.
   (b) Time required for demonstrating.
   (c) Space and electric points required.
   (d) Anything else required, including microscopes.

A comparative exhibition of requisition and report forms is suggested. *Don't just talk about that special gadget, bring it!*

In regard to the actual timetable it is intended that Thursday evening will be used to complete the business not dealt with in the morning, and possibly for a symposium. Time will also be made available for papers not falling under any of the above headings.

Your comments are invited.

BOB ALLAN,
Conference Secretary,
Pathology Department,
Medical School, Dunedin.

The Jubilee of the Pathology Department, Christchurch Hospital.

D. H. ADAMSON
Principal Bacteriologist.

(Received for publication, December 1962.)

Last year was celebrated the fiftieth anniversary of the opening on July 16, 1912, of the Pathology Department at Christchurch Hospital by Dr A. B. Pearson.

A retrospective exhibition of laboratory equipment, medical literature and laboratory reports was opened at a morning tea provided by the North Canterbury Hospital Board on Monday, July 16, 1962. This was attended by about fifty hospital medical staff, specialists and senior laboratory staff, and was held in a large room in the Blood Bank.

The exhibition, set up chiefly by Dr D. T. Stewart, the present Director, included glimpses of laboratory work in Christchurch during the last century, foundations of medical laboratory work and its development here. On display were copies of the first reports issued, microscopes and microtomes nearly 100 years old, waterbaths, incubators, colorimeters and other equipment used in the department during the early days. Milk bottles and other improvised equipment used for the first Blood Bank in 1939 were also exhibited.

The progress of the department throughout its life was vividly displayed on sheets of softboard by chronological notes, photographs, plans and by lists of staff for each of the fifty years — beginning with one and finishing with seventy-two members. This display was afterwards transferred to the Pathology Museum, where it remained open to hospital staff and friends for many weeks.

Conducted tours of the Pathology Departments at both Christchurch and Princess Margaret Hospitals were arranged, and afternoon tea for all past and present staff members was provided by the Board, at which about 100 visitors attended, all joyful at meeting old friends and most enthusiastic in their praise of the exhibition.

An official Dinner was held in the evening, when toasts were given to "The Pathology Service," "Our Guests" and "Past Staff." Over one hundred past and present staff members and invited guests were in attendance.

THE JUNIOR ESSAY COMPETITION

Each year, a prize of £5 5s 0d is awarded for the best entry in each of the two sections of the Junior Essay Competition.

TECHNICAL SECTION: Descriptions of methods or techniques in use in the laboratory.

ESSAY SECTION: Essays on general or historical aspects of medical laboratory technology.

All trainees are eligible to enter.

Intending entrants should study the "Directions for Contributors" appearing on the inside front cover of this issue of the Journal, as this mode of presentation should be followed as closely as possible. They should indicate for which section of the Competition they wish to enter, and give their name and address on a separate piece of paper.

Essays should be submitted to the Editor of this Journal before June 21, 1963.

Letters to the Editor

CONFERENCE BUSINESS

Dear Sir,—The last Annual Conference seems to have been a fiasco for many because the so-called business section took up much of the available time. Perhaps some of your readers would comment on some suggestions which might help to overcome this without encroaching on the first evening usually put aside for a tour of the local laboratory—always of interest and often of much value.

As is well known, papers are not easily forthcoming and much time goes into their preparation, so it is disappointing to hear them read at top speed in order to get them all into what is left of the time.

Following on experience gained from the last Conference, it would seem that some of the remits were not sufficiently prepared by the Branch responsible before being submitted. If they have to be drastically re-worded as some were (to the sound of much noisy "thought") before being discussed, then the chairman should have the power to return them for redrafting and presentation the following year. No purpose is served by circulating a remit which is then altered at the meeting, especially as those carrying proxy votes cannot legitimately use them. This, of course, refers to the first introduction of a remit and has nothing to do with motions and amendments.

Secondly, too many people got up to speak with only a very vague idea of what they wanted to say, and time was wasted while they thought on their feet. I suggest that a definite time limit be placed on all speakers—say two minutes for those introducing a remit, and one minute for those wishing to speak to it. The chairman would have the right to extend the time if he felt it was worthwhile.

Thirdly, the same thing was said far too often in a different way. If a speaker wishes to agree, he should say so and sit down. I admit that this is very difficult to control, but I think that all members should be reminded from time to time, together with the bit that goes, "I don't know anything about this but my view is . . ."

These suggestions are in no way offered as a criticism of the chairman, who had my sympathy, but are offered in the hope that some formula may be found to cut business to a minimum, and have as much time as possible for papers, discussions and tours of technical interest.

I am aware that a lot of the business is vital to the Institute and requires investigation, but I think we should trust our Council more than some of us appear to do, and listen to our own voices a lot less.

P. H. CURTIS,
19th September, 1962.

EXAMINATION QUESTIONS

Dear Sir,—In the October Intermediate Examination, a series of a type of multiple selection question appeared for the first time. This style of question is gaining popularity in examinations these days and I suppose it was inevitable that it would crop up sooner or later in our Health Department's examinations for technologists. I would not attempt to deny the usefulness of questions like them, but I really must protest at the confusion which it must have caused in the minds of candidates at this particular examination.

The paper consisted of 40 brief questions followed by four possible answers which were lettered from (a) to (d). Examinees were given a special answer sheet and were instructed to place a cross under the appropriate letter *(singular)* to indicate which of the four answers they

considered to be correct for each question. No indication was given that in some cases more than one of the alternatives could be equally correct, and naturally, candidates will have wondered whether they were expected to place a cross under *more* than one letter or to abide rigidly by the instructions and risk losing marks by not appearing to know that more than one of the answers were right. I have to confess that on being shown the paper afterwards, I felt that I would have wondered, too.

I daresay that your April issue will contain a reproduction of the paper, and your readers may wish to refer in particular to questions 16 and 17 to see if I am not correct. Furthermore, as I see it, in question 33, *none* of the four answers is correct, for my definition of "titre" is that it is the reciprocal of the *highest* dilution which will cause agglutination of an antibody with its corresponding antigen.

I hope that I am not appearing hypercritical of the Examination Board, but as one who has the responsibility for preparing trainees for the examinations, I do feel that it is a pity if they lose even a very few marks because the questions are confusingly worded.

MENTOR (name and address supplied),
21st January, 1963.

CHANGES OF ADDRESS

The Institute mailing list is maintained by the Journal Committee who have charge of the addressograph plates.

Members who change their mailing address or (in the case of female members) their name, should notify the Editor of the Journal without delay. The information is then automatically passed on to the Secretary and Treasurer.

Members wishing to complain of non-receipt of Journals and correspondence should write to the Editor, quoting their current subscription receipt number.

THE REX AITKEN MEMORIAL PRIZE

Members are reminded that this award of £5 5s 0d is made annually through the generosity of Biological Laboratories Ltd. of Auckland.

Competition for the prize is open to all members of the Institute who have published an article of a technical or practical character, in any periodical, during the year 1962.

Intending entrants should submit 3 copies or reprints of their work to the Editor of this Journal before May 31, 1963.

VACANCY

9/63/359: TECHNICIAN, AGRICULTURE DEPARTMENT, RUAKURA ANIMAL RESEARCH STATION.

Salary: Up to £1,085 a year.

Qualifications desired: New Zealand Hospital Laboratory Certificate of Proficiency, F.I.M.L.T., or equivalent qualifications.

Duties: Technical Bacteriological duties in a Salmonella Investigation Unit.

Applications close on 1st May 1963 with the Secretary, State Services Commission, P.O. Box 8004, Wellington. Use form SSC 17A obtainable at Post Offices, enclose copies only of testimonials and quote Vacancy No.

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