THE NEW ZEALAND JOURNAL OF MEDICAL LABORATORY TECHNOLOGY

THE OFFICIAL PUBLICATION OF THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY INCORPORATED.

VOLUME 18. No. 1 APRIL, 1964

Directions for Contributors

In the interests of uniformity, the following directions should be followed by all contributors to the Journal.

Manuscripts should be typewritten on one side only, of good quality quarto sized paper, be double spaced and with a 1½in margin. They should bear the author's name (male authors give initials, female authors one given name), address, and (if this is different), the address of the laboratory where the work was carried out. The manuscript should take the following form: Introduction; materials and methods; results; discussion and conclusion; summary; acknowledgments; references. Carbon copies are not acceptable. Nothing should be underlined unless it is necessary that it be printed in italics.

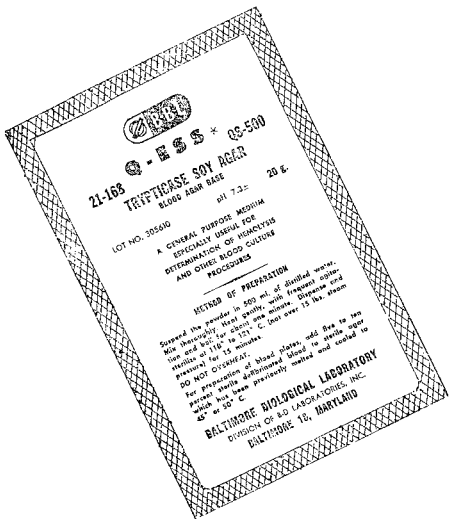
ILLUSTRATIONS. Graphs and diagrams are termed 'figures' and should be numbered in the order of their appearance in the text. Figures should be drawn in Indian ink on stout white paper larger than required for the text. Legends to the figures should be typed separately and attached. Illustrations, particularly half tone blocks, should be sparingly used. Half tone blocks are referred to as 'plates' and these again are numbered in sequence and the captions are typed and attached. Elaborate tables should be kept to a minimum but any necessary tables should be typed on separate sheets of paper and numbered in roman numerals.

NOMENCLATURE. Scientific names of micro-organisms should conform with the system adopted in the latest edition of *Bergey's Manual of Determinative Bacteriology* and underlined to indicate that they are to be printed in italics. Collective names for groups of bacteria such as staphylococci, salmonellae, etc., should not be underlined. Abbreviations such as C.S.F. for cerebro-spinal fluid, are only permissible if their meaning is clearly indicated when first introduced. Conventional abbreviations such as ml. for millilitre and cmm. for cubic millimetre are acceptable without explanation. Names of chemical substances should conform to current chemical practice and care should be taken to see that chemical formulae are correct.

REFERENCES: Only papers closely related to the author's work should be quoted. Contributors should study this issue of the Journal for examples of the preferred method of making reference. All references are brought together at the end in alphabetical order and numbered. In the list, references should include (1) Surname followed by the initials of the author(s), (2) Year of publication in brackets (3) Abbreviated title of the periodical according to *World List of Scientific Periodicals* or to *World Medical Periodicals* (underlined), (4) Volume, (5) Page numbers. If there are three or more authors the words *et al* may be added to the name of the first author in the text, but the names of all co-authors must be given in the list. References to books should include (1) Author(s) or Editor(s), (2) Year of publication in brackets, (3) Title (underlined), (4) Edition, (5) Page number referred to, (6) Name of Publisher and Place of Publication.

REPRINTS. Authors may receive a minimum of 50 reprints at cost price. These should be ordered when returning corrected galley proofs.

PROOFS. Whenever time permits, authors will have the opportunity to correct galley proofs before publication. No major alterations will be permitted unless the author is prepared to stand the cost, and proofs must be returned within three days of receipt.



Q-ESS

PRE-WEIGHED, FOIL-PACKAGED, DEHYDRATED CULTURE MEDIA

PRE-WEIGHED QUANTITIES OF POWDER, SEALED UNTIL THE MOMENT OF USE, IN LIGHT AND MOISTURE - RESISTANT FOIL ENVELOPES EACH CONTAINING A QUANTITY SUFFICIENT TO MAKE A SPECIFIC VOLUME OF FINISHED MEDIUM.

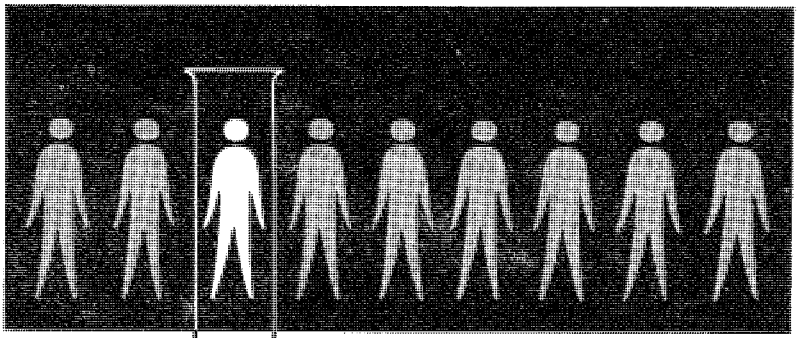
Features:

1. **EXACT WEIGHTS FOR CONVENTIONAL VOLUMES . . .**
   Eliminates Accidental Errors.
2. **SEALED TILL THE MOMENT OF USE . . .**
   Just Snip or Tear Open, and Use.
3. **EFFECTIVE LIGHT BARRIER . . .**
   No Degradation of Nutritional Components.
4. **EFFECTIVE MOISTURE BARRIER . . .**
   No Changes in Bulk Density Due to Ambient Humidities.
   No Caking — Powder at Optimum Conditions for All Requirements.
   Nutritional Value Maintained.
5. **ELIMINATES WEIGHING, MANY STEPS AND USE OF EQUIPMENT OR ACCESSORIES.**
6. **A PRECISE, RAPID, TIMESAVING PROCEDURE . . .**
   Instructions on Each Envelope.
7. **AN INVENTORY SAVER ON MEDIA INFREQUENTLY USED.**
8. **A PREREQUISITE FOR MEDIA USED DAILY . . .**
   Significantly Multiplies Savings of Time and Benefits Secured From Reducing Spoilage and Waste. Provides Media of Uniform Characteristics and Maximum Performance.

Sole Agents for Baltimore Biological Laboratories:

BIOLOGICAL LABORATORIES LTD.

Woodside Avenue, Northcote.
P.O. Box 2749, Auckland, C.1.
Telephones 18-125 and 19-748.



to detect bleeders before surgery

a new tool for new dependability

Platelin™

standardized platelet factor reagent

Newer tests have been devised to replace the Lee-White Clotting Time, which is known to miss nearly 50% of proven hemophilia cases.(1). These tests are far more dependable indicators of potential bleeders than the routine bleeding and clotting times. They are relatively rapid and simple. But they require a rigidly standardized platelet factor reagent. Until now, no such reagent has been readily available.

PLATELIN is platelet factor reagent, rigidly standardized against normal plasma, against DIAGNOSTIC PLASMA Warner-Chilcott and against plasmas deficient in stage one clotting factors.

Using PLATELIN, any worker skilled in the technique of the Quick One-stage Prothrombin Time can rapidly perform either of two new and sensitive coagulation screening tests:

The Hicks-Pitney Test(2)—a rapid, simplified screening version of the Thromboplastin Generation Test, especially adapted for routine use, which duplicates the extreme sensitivity of the TGT. The only commercial reagents needed are PLATELIN and DIAGNOSTIC PLASMA Warner-Chilcott.*

The Partial Thromboplastin Time Test(3) — similar in procedure to the One-stage Prothrombin Time, and approximately equal to the Prothrombin Consumption Test in detecting bleeders. Only one commercial reagent is required: PLATELIN.

Complete directions for performing and interpreting both the Hicks-Pitney and the Partial Thromboplastin Time tests are included with each package of PLATELIN.

Take advantage of this new advance in coagulation research. Order a supply of PLATELIN today.

Each vial of PLATELIN is sufficient for 12 Hicks-Pitney or 25 Partial Thromboplastin Time tests.

Boxes of 10 vials, 2.5 ml. size, 60/-.

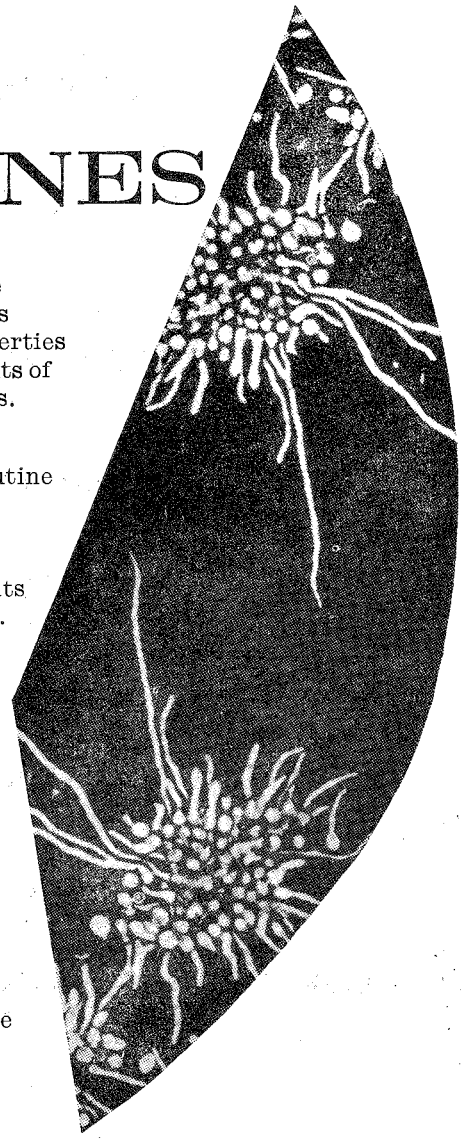
References: 1. Wilkinson, J. F.; Nour-Eldin, F.; Israels, M. C. G., and Barrett, K. E.: **Lancet** 2:947 (Oct. 28) 1961.
2. Hicks, N. D., and Pitney, W. R.: **Brit. J. Haem.** 3:277, 1957.
3. Langdell, R. D.; Wagner, R. H., and Brinkhouse, K. M.: **J. Lab. & Clin. Med.** 41:637, 1953.

* In addition to its use as a **reagent** in the Hicks-Pitney test, DIAGNOSTIC PLASMA Warner-Chilcott remains the **normal plasma of choice for quality control** of the one-stage prothrombin time and other coagulation tests. Make sure your supply of DIAGNOSTIC PLASMA Warner-Chilcott is adequate.

William R. WARNER and Co Ltd
P.O. Box 430, Auckland

OMNIS ORBIS
WARNER

OXOID PEPTONES



The Oxoid Laboratories have developed a range of peptones differing widely in their properties so as to meet the requirements of workers in many varied fields.

OXOID PEPTONE L37
is used extensively for all routine purposes in laboratories throughout the world. It is a high-quality peptone which gives consistently good results with standard culture media.

OXOID PROTEOSE PEPTONE L46,
a more specialized peptone, is prepared by the papain digestion of selected fresh meat, for use in media for the production of bacterial toxins.

OTHER OXOID PEPTONES AND HYDROLYSATES:
Peptone L6, Tryptose, Soya Peptone, Mycological Peptone, Tryptone, Tryptone "T," Casein Hydrolysate (Acid), Peptone "P," Liver Digest Peptonized Milk.

For progressive 'aboratories . . . OXOID culture media

MANUFACTURED BY OXO LTD., LONDON

Sole New Zealand Agent Edwin A. Piper Ltd., 4 Rata Road, Cheltenham, Auckland, N.1.
Telephone 70-040. **Telegraphic address:** "Eapagent" Auckland.

THE NEW ZEALAND JOURNAL OF MEDICAL LABORATORY TECHNOLOGY

VOLUME 18. No. 1. APRIL, 1964.

The Journal is published thrice yearly (in April, July and October) and is distributed without charge to all financial members of the N.Z.I.M.L.T. (Inc.). Subscription to the Journal for non-members is ten shillings per year or four shillings per single issue postage paid.

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.

Enquiries regarding advertising rates should be addressed to the Advertising Manager at the Microbiology Department, Medical School, Dunedin.

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

Status and Training

Delegates who attended the 1963 Annual Conference in Dunedin will not readily forget the forum on Status and Training that took place on the Thursday afternoon. A guest chairman of no mean perspicacity, Mr K. H. Melvin, of the University of Otago Education Department, was swift to grasp and evaluate the salient points of the situation; even swifter to demolish delusions of grandeur with his highly pertinent observations relating to aims and aspirations for the future of medical laboratory technology. The ovation he received at the conclusion of the forum testified to the lack of any rancour resulting from shattered illusions, and served as a measure of the Institute's gratitude for the glimpse of harsh reality which Mr Melvin's remarks had imparted.

It is not difficult to fall into the error of becoming emotional about so vital a question as status, so as a blueprint for future action and for present sober consideration, transcripts of Mr Melvin's opening address and concluding summary are here presented.

INTRODUCTION

This afternoon's session has been set down as a forum on the topic, 'The Status and Training of Medical Laboratory Technologists.' By way of introduction my task is to set the pattern whole and to suggest that, however preoccupied you may be with your particular part of this national — indeed universal problem of training and status, you are reassured that every learned body, every technological body and technical body of any consequence in the world today, is exercised with precisely this your problem.

Now the first point to be made is that your mentors, in drawing this topic, have rightly seen that status and training are complementary and correlative; each bears directly upon the other. To hammer the obvious point, may I say that if we keep in mind throughout our discussions that our status as a profession or semi-profession will depend entirely upon the quality and quantity of our training, so that status and training are rightly posed in the topic this afternoon, as twin legs upon which the Institute must walk.

The second point I think worth making, is that technology is exceedingly difficult, in this country, to define as separate from the technical aspects of both the professions and industry. A little later we shall have occasion to refer to the definitions laid down by the statutory bodies in this country for both of these creatures; but if you ask yourself, 'In the second case do I know clearly what is the definition of a technician as distinct from a technologist,

and am I quite certain that I belong to the one and not the other,' I think that your mental set will be helpful to the discussion.

The third point is that if we accept as a rough approximation that there are ascending stages of competence, status and public acceptance from the technician to the technologist, we ought to realise that the field of training for technologists in New Zealand has been traditionally the role of the University. For a variety of reasons even the separate and autonomous universities, as we now have them, are as reluctant as was the original University of New Zealand and its constituent colleges, to add to its technological responsibilities. You are familiar with the fact that the Universities variously cater for such technologies as Accountancy, Engineering, Surveying and Architecture. The members of these would regard themselves very definitely as professions, but from the point of view of the University these are technologies. There is a mediaeval emphasis, particularly in our University of Otago — God preserve it — which views askance the entry of these upstart disciplines; and in this we are not alone for we are tending in this country, as always, to follow the English precedent. In England, in a desire to preserve sanity amongst the multiplying universities, they have embarked upon the development of tertiary education through what we call CATS, Colleges of Advanced Technology. Now these colleges, mark you, will not sit in the Pantheon of Education with the true universities; they are set apart. But by reason of national need and institutional pride, they are establishing themselves as the equivalent of universities — but geared to this century rather than, shall we say, the University of Paris in the year 1200. There is, in other words, a reluctant recognition by Authority — the State — that in our modern needs we must go beyond the universities into proliferating tertiary institutions; and Colleges of Advanced Technology are England's answer.

We have a somewhat similar situation in this country. The White Paper of 1956 was inappropriately called 'Technical Education' because they didn't even know then how to distinguish between the two. But this White Paper of '56 laid down the rationale of technological institutes outside the university orbit, yet placing them with it in status, depth and breadth of course training. This makes it clear that these new institutions must examine very carefully their own training programme and the status they aim at. Now is this not precisely where you find yourselves ? We have in this country a new-born fledgling: the Central Institute of Technology, with two subordinate institutes. It has already established something of its future character by absorbing the College of Pharmacy. The pharmacist will regard himself as 'profession,' but from the university point of view he is 'technology.' One of the questions that I think you might address yourself to, is whether you can, at this stage of your

evolution, rightly foot it with the technologies of architecture, surveying, accountancy and pharmacy; for these are technologies in the strict thought of the Government, under whose ample umbrella we must all take shelter.

However, there is a note of hope injected with the 1962 Commission on Education, that enormous volume of which the page reference is a bedside book for the next decade. This urged that the new tertiary bodies that are emerging in this country, tentatively following the English pattern but undriven by any great industrialisation, any great population drive, or any of those urgencies that you see paramount in continental countries; unaccompanied by that, this is a country that has tried to move into a similar pattern, and the 1962 Commission counselled caution of these new bodies in their natural desire for autonomy. It was again the ancient aphorism: walk before you run.

This is also before the forum this afternoon, as to which of two alternatives, (I take it that you have resolved that the third is out); that which two alternatives — which are successive stages of autonomy — you ought to favour. You may only favour it, for you must supplicate the gods. If you choose the right one, which has a chance of being accepted by the Government and instituted by statute, then you are facing the caution of this 1962 Commission. Now what do they have in mind ? That before your Institute — or any other — has a chance of satisfying the State as to your integrity and your capacity to run your own affairs, certainly before you can win the esteem you desire from the pathologists and the medical profession generally, and ultimately before you can hope to mobilise the public confidence which lies behind your financial reward — because you know the Government does not pay out unless the public wish it — then these four stages of thought are all part of your problem. Are you at a stage where you can sensibly ask for autonomy ? And to what degree ? This was the 1962 Commission's advice. Now I think that is about all I ought to say, having an eye on the enemy — time.

You will realise that the Secretary and his committee have desired that this shall be a forum. If I have, then, sketched broadly the pattern of evolution of tertiary education and the emerging pattern of institute training—I have nothing to say as to when you are going to get it or to what degree you are going to get it, you will know better than I how it should be done — but if this broad pattern offers you some kind of conspectus, we shall proceed now to the forum. My only last thought is before we get down to detail, and your secretary has, very sagely if I might say so, designed the discussion in successive stages of depth. The first will be preliminary and we hope to end profoundly, but before you embark on this, might I, as an outsider,

commend to you what we have adopted for the teaching profession, which is precisely in your situation of suspended animation or arrested development. There will be no joy in our Canaan until we are a graduate profession; none at all! There is no reflection upon the founders of this Institute if you are not already a graduate profession. Dr D'Ath reminded us this morning that in a single generation you have come from nothing to something: troubled by mushroom growth. Therefore it may not be an immediate quest; but I would commend to you from the experience of institutes of technology all over the world and the professions themselves, that before committing yourselves to any immediate measure of autonomy, or organisation, or statutory government, you will see that the ultimate door is kept open to becoming a graduate profession. If you do this, then I feel that our forum may satisfy your immediate needs and the ultimate aims and aspirations of your own technological institute.

SUMMING UP

The present situation, as I have understood it from your discussion, is that in a single generation you are now reaching for professional status. The first official reaction to that has been one of a not unnatural caution on the part of the Department. There is a suggestion of a new Board to replace the old bifurcated one, preponderantly outer-directed rather than inner-directed. This I think you must recognise as inevitable in the face of our internal disarray. The third point is that the Institute is, in fact, able to mobilise a certain working competence; but is this an adequate answer to the tacit doubt as to Institute strength for professional status and autonomous control? The training programme in New Zealand is not comparable, as yet, with that of technology generally, and overseas medical laboratory technology in particular. Can these training programmes be improved, here and now, to cover, immediately the status sought; or have we unfinished business within ourselves; our own ranks and organisation to attend to before we ask for anything more? Mr Allan has shown how much in the main centres is mere technician's work under outer professional direction, and the tacit implication is either that there is a doubt of those who employ us as to our capacity, or an impossibility on our part to do more than routine demands of us. Mr Kennedy showed how correspondence tuition is invoked because either charge technologists do not teach enough, or teach inadequately, or the initial training is too scant for a man to know how to teach himself thereafter, or else we are too busy for teaching and so **good** does duty for **best.** Also, where a correspondence tuition dependent sole charge laboratory finds it is even more difficult to find time for in-service training, (a) because there are too many varieties of jobs to be

done in the sole charge set up, and (b) there are too few people to do it and teach. The worst thing about this is that places that do qualify numerically for a tutor haven't all got one. A tutor technologist seems to be engaged on the numbers of trainees in a ratio that may well be archaic and ought perhaps to be challenged. One tutor to thirty trainees may not be at all adequate, but you will not get that situation improved until everyone entitled has a tutor. 'Every man is a debtor to his profession' said Bacon, so there is an obvious need for dedication amongst you qualified people, for something beyond the call of duty in tutoring your trainees. But if this, in fact, should be done by full-time tutors if it is a statutory teaching situation, why should you attempt to carry it beyond the point of efficiency? Should you not ask the authorities to tidy up the tutor situation to an orderly plan, if not decided by yourselves then determined by the Department? Mr Horner felt that we were reaching towards a university-recognised and oriented graduate course, as the only satisfactory basis for both status and training. I think he is on the side of the angels, but perhaps two or three decades before his time. I hope not; I hope he is right and I am wrong. The immediate step is that a new Board is to be appointed, but I cannot see, ladies and gentlemen, with the utmost goodwill in the world, that you are in a position to challenge anything that the Department proposes to do with you; and if this is the real nub of our case, then I think we have not been wasting our time. A council of war has shown that we are in no kind of position to declare war on anyone. If you can, therefore, empower your incoming president and executive to pursue the constructive points of this discussion, and such others as emerge from your Conference, with all speed but with all reality, shall we say actuality, then I think that is all we can hope to do. Therefore I have very great pleasure in thanking you for your contributions, in hoping that all is not inspissated gloom, and that you can go out feeling that there is still life because there is still hope.

International Association of Medical Laboratory Technologists

The International Congress of Medical Laboratory Technologists will be held between June 15 and June 19, 1964, at Lausanne, Switzerland.

Any person interested in attending the congress should write directly to the Secretary of the I.A.M.L.T., Miss E. Pletscher, Frauenklinik, Zurich 6, Switzerland, who will supply further details regarding registration, programme and accommodation. The registration fee is Swiss Fr. 70. (approx. £6).

Canadian Technologists

In the guest editorial *What of the Future?* published in the October 1963 issue of the *Journal,* it was stated that a Canadian trainee medical laboratory technologist takes a full-time course of instruction for a period of two years before sitting the Licenciate Examination, and that a higher specialist qualification for Advanced Registered Technologist is also available.

The Canadian Society of Laboratory Technologists has pointed out that these statements are erroneous and misleading, and that the Licenciate Examination represents, in fact, the most advanced form of qualification in medical technology in force in Canada at the present time.

As a point of interest, the system of certification of technologists in Canada works in the following way:—

Entrants must have a minimum academic standing, the term applied to which varies in different provinces, but which is broadly stated as Senior Matriculation or University Entrance level.

Once accepted, the trainee undergoes a course of teaching which may vary from the minimum of eighteen months, to twenty-four months, and which may take any one of five forms:

1. *Individual Hospital Approved Centres,* in which hospitals approved by the Canadian Medical Association and the Canadian Society of Laboratory Technologists accept and undertake the training of their own students. The course is generally of eighteen months duration, and includes both formal lectures and practical training.
2. *Centralised Training Programmes,* in which several hospitals combine their facilities to provide training for students within the group. Each hospital accepts its own quota of trainees, but these are sent to one hospital which is equipped to provide lectures and bench training. After the first year, the student returns to his or her parent hospital for a second year of practical instruction and experience.
3. *Courses in Technical Institutes,* which are of limited availability owing to the fact that this form of training is of recent introduction and is still in the process of development. In co-operation with the Canadian Society of Laboratory Technologists, these institutes are providing the instruction necessary for certification. After one year at the Institute, the student proceeds to an approved hospital centre for practical training.
4. *Diploma Courses at Universities,* which are essentially the same as those provided at technical institutes.
5. *University Courses Leading to a Degree,* in which the student receives practical training in an approved hospital during the summer months or during the last year of the university programme.

Whichever of these types of training programme the student enters, he or she is required to complete no less than 12-18 months practical training in an approved hospital before sitting the qualifying examination of the Canadian Society of Laboratory Technologists, which entitles successful candidates to the initial certificate of Registered Technologist (R.T.) of the Society.

Following initial qualification, the technologist may then proceed to Advanced Certification level (A.R.T.) after at least three more years of training and experience, which may be either general or specialised.

At length, after a minimum of yet another three years' training, the A.R.T. may elect to present himself for examination for licentiate certification (L.C.S.L.T.). Again, this examination may be taken in one specialised subject or in the general field (which includes laboratory

organisation and administration). Candidates are subjected to a searching test of their capabilities, and are required to demonstrate their competence to instruct, their conversance with the literature and their ability to handle the responsibility of managing a laboratory or a department. Thus, a holder of this most advanced qualification must have, at the very least, eight years experience; in addition to the capacity to pass an examination for which there is no limiting formal syllabus. He must be abreast of current developments in his field, competent to handle the sort of technical and administrational crises that arise in every laboratory from time to time, as well as being able to cope with quality control and the evaluation and implementation of new techniques.

On the face of it, the R.T. would appear to be at a level *below* that of the C.O.P., but the L.C.S.L.T. is clearly a technologist supreme and not, as we have regrettably led our readers to believe, a person with the barest minimum right to call himself a technologist.

If any technologist contemplating emigration to Canada requires information regarding educational requirements and certification procedure there, they should get in touch with the Canadian Society of Laboratory Technologists at 99 Wentworth Street South, Hamilton, Ontario, who have kindly volunteered to supply such details to anyone who wants them.

Total Serum Cholesterol Estimation

A Review of Literature — Investigation of a Current Technique — Several Methods Compared — Study of a New Method

E. K. FLETCHER

Chemical Pathology Laboratory, Pathology Department, Medical School, Dunedin.

(A paper read at the 1963 Conference of the N.Z.I.M.L.T.)

Recent interest in serum cholesterol procedures has resulted in many important observations. Particular notice has been drawn to the methods employing an acetic acid solvent and ferric chloride colour reagent[16 17]. Bowman and Wolf (1962)[3] show an absorbance peak at 490mμ, as well as the expected 560mμ peak, using glacial acetic acid which had not been redistilled. Moore and Boyle (1963)[8] give evidence of errors concerning the direct determination with a ferric chloride colour reagent. Generally, this lack of specificity is attributed to the reaction of protein-bound tryptophan and the aldehydes of impure acetic acid used as a solvent[3 8]. Also, recent surveys indicate a measure of dissatisfaction with current serum cholesterol methods. Tonks (1963)[14] distributed a serum of known cholesterol content to one hundred and seventy laboratories and received values of 34% error on the mean by the Zak (1957)[16] method, as compared with 15% error on the mean by the method of Schoenheimer and Sperry (1934)[11]. Inquiries to two hundred and seventy workers by Rice and Grogan (1962)[9] revealed the employment of more than sixteen different serum cholesterol methods; that of Bloor (1916)[2], using the unstable Lieberman-Burchard colour reaction, was the most popular, being the choice of 21% of workers.

Routine cholesterol estimations have been practised in our laboratory by the method of Zak[16] for the past three years. It became evident from the above reports and high values obtained, that our method should be investigated and, ideally, one of greater specificity adopted.

Total serum cholesterol estimations were performed on one hundred apparently healthy, non-hospitalised individuals by the method of Zak[16]. Ages of the subjects varied from fifteen to sixty-two years, and there was no significant variation between males and females used in the investigation. Fig. 1 shows the percentage population for cholesterol values, and the distribution as being significant and normal. The mean total cholesterol level was

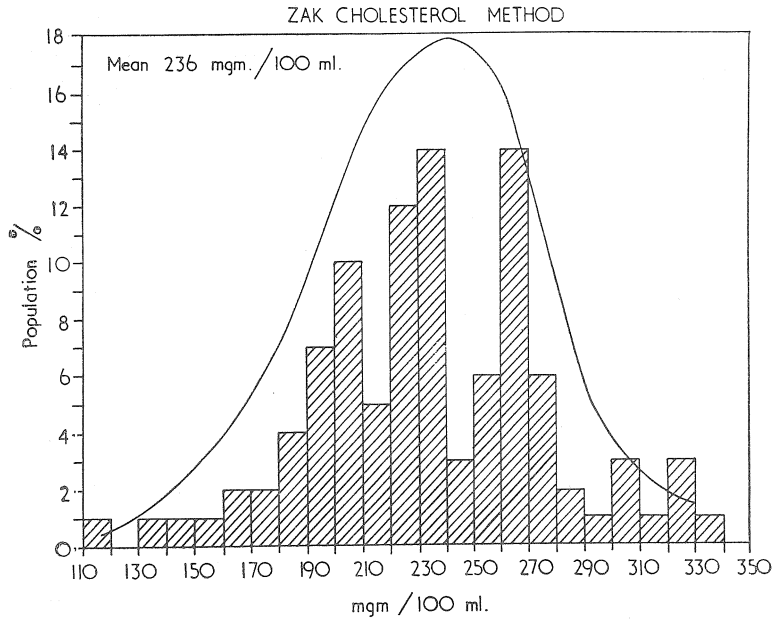


Fig. 1. Distribution of cholesterol values by the Zak method from one hundred normal individuals.

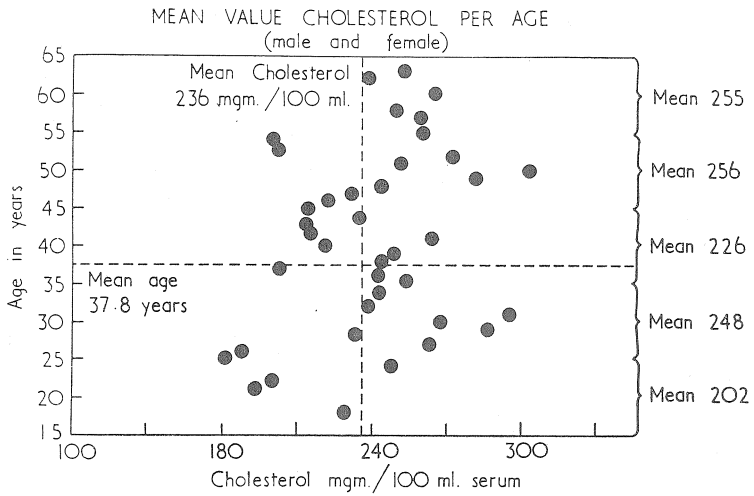


Fig. 2. The same population as in Fig. 1, illustrated to show the distribution by age. Mean values at the right-hand edge represent the mean for each ten years of age.

found to be 236 mg. per 100 ml., and the statistical normal range* 153 to 319 mg. per 100 ml. Fig. 2 illustrates the distribution of mean cholesterol value by age. To compare the above method, that of Trinder (1952)[15] was employed and, in all, thirty-four estimations on the above subjects evaluated. Fig. 3 shows comparative distribution by both methods. The mean value by Zak was 231 mg. per 100 ml., and by Trinder, 193 mg. per 100 ml.

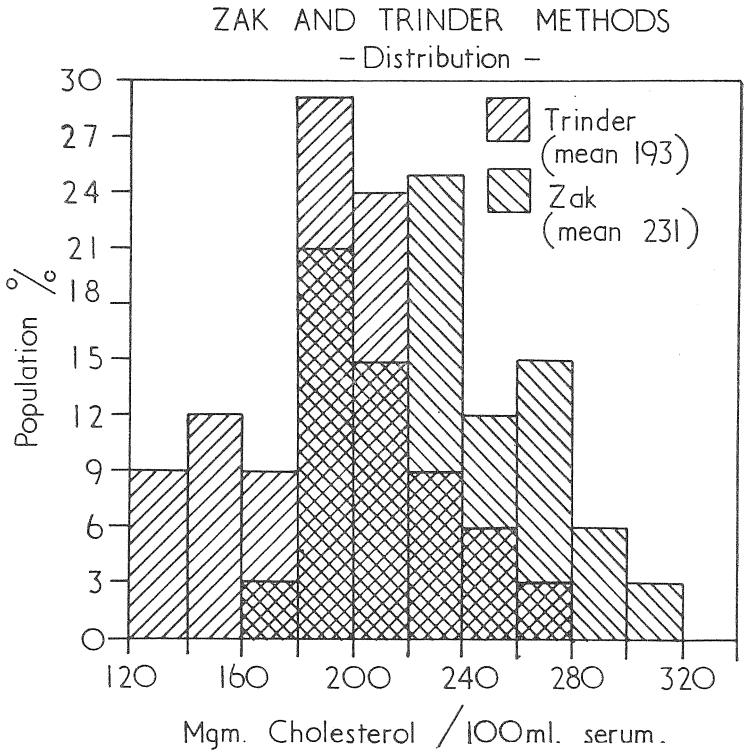


Fig. 3. A comparative histogram showing the lower values obtained from the same population by Trinder's method.

The results, on comparison, revealed a marked lack of correlation; however, the method of Trinder[15], being a modification of earlier work[1 11], would experimentally appear to be more specific.

Further Studies:

In order to make a more accurate assessment of our current method, two samples of pooled huma sera and one of a commercially-prepared serum were processed by the Zak[16], Trinder[15], Sperry and Webb[12], Bowman and Wolf[4] and Leffler[6]

* Formula: normal range = mean $\pm$ (1.96 x standard deviation).

(as described later) techniques. A standard, equivalent to 200 mg. per 100 ml., used in all methods, proved to be accurate within 3%. Table I shows comparison of serum cholesterol concentrations by the respective techniques, most striking being the higher values by 10% or more of the Zak method, confirming previous results.

COMPARISON OF METHODS

Method	Mgm. Cholesterol 100ml. serum Sample "A"	Sample "B"	Control* (90mgm./100ml.)
Trinder - - -	220	256	111
Sperry-Webb -	219	265	97
Bowman-Wolf	222	281	101
Leffler - - - -	223	267	95
Zak - - - - -	246	292	119

Each value shown is the mean of three estimations.
* Sample containing 5mgms. Bilirubin /100ml.

Table I. Cholesterol values of two samples of pooled sera, and a control serum compared by five different techniques.

The widely recognised methods of specificity, Schoenheimer and Sperry[11], Sperry and Webb[12] and Trinder[15] are unsuitable for routine laboratory use where several specimens are processed every day. They are time-consuming, utilise many and large quantities of reagents, and involve many manipulations. Both Leffler[6] and Bowman and Wolf[4] employ the Rosenthal *et al.* (1957)[10] colour reagent, while isopropyl alcohol and ethanol respectively, are used for protein precipitation and cholesterol extraction. These two techniques are rapid, simple, and require only a small amount of serum. Because results by the method of Leffler[6] (as described later) were consistent and reproducible, this was the method adopted for further investigation.

Proposed Method for Total Serum Cholesterol:

Essentially, this is that of Leffler[6], but with modifications found necessary by experiment in our laboratory. Isopropyl alcohol is added to serum, producing a finely divided protein precipitate and at the same time extracting cholesterol. After briefly shaking and standing, the mixture is centrifuged and an aliquot of the clear supernatant taken for the addition of colour reagent. The resultant purple solution is read photo-electrically

after 15 minutes. It has been proposed that the colour reagent of Rosenthal *et al.*[10] is more satisfactory than previous developments[13] and that isopropyl alcohol is an excellent extracting reagent, giving total extraction in a short time at room temperature and a low blank density[13]. By using a greater volume of serum and isopropyl alcohol this method is readily adaptable to the ester cholesterol estimation and by relative variation of reagents is suitable for micro technique employing 0.05 ml. of serum. In these studies 0.1 ml. volumes of sera were used.

Experimental

Although time for colour development was originally published at ten minutes, stability was found only after fifteen minutes. Thereafter density readings continued for one hour showed no change. Density readings as seen in Fig. 4 show

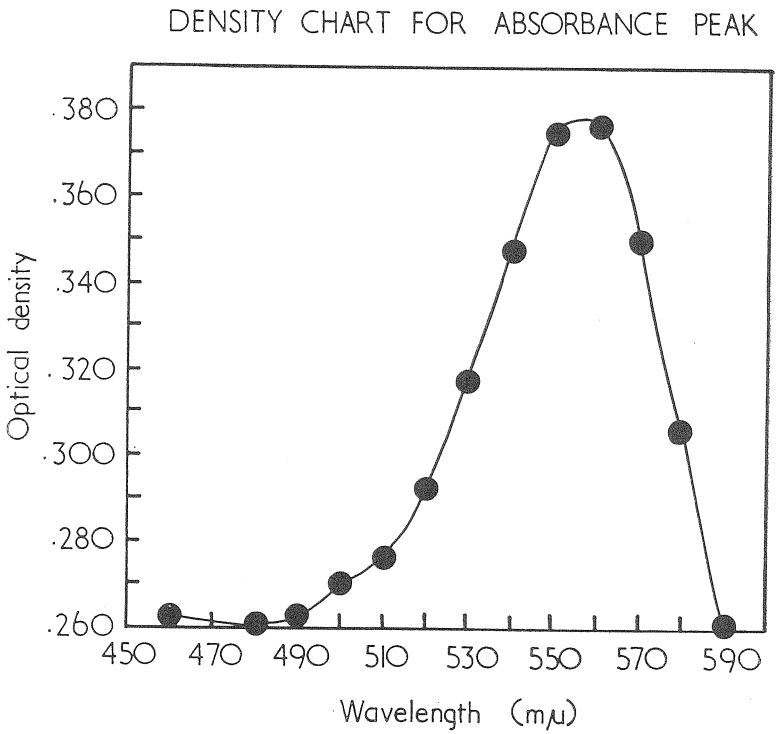


Fig. 4. Absorbance peak of 80μg. cholesterol by the modified Rosenthal colour reaction.

maximum absorption at 560mμ. These were determined with a 0.08 mg. per ml. cholesterol concentration between 470mμ and 590mμ wavelengths. The absence of a second interfering

peak at 490mμ is noted. Colour reagent was added to one ml. volumes of varying concentrations of cholesterol in isopropyl alcohol (Fig. 5) and densities for 100 mg. to 400 mg. per 100

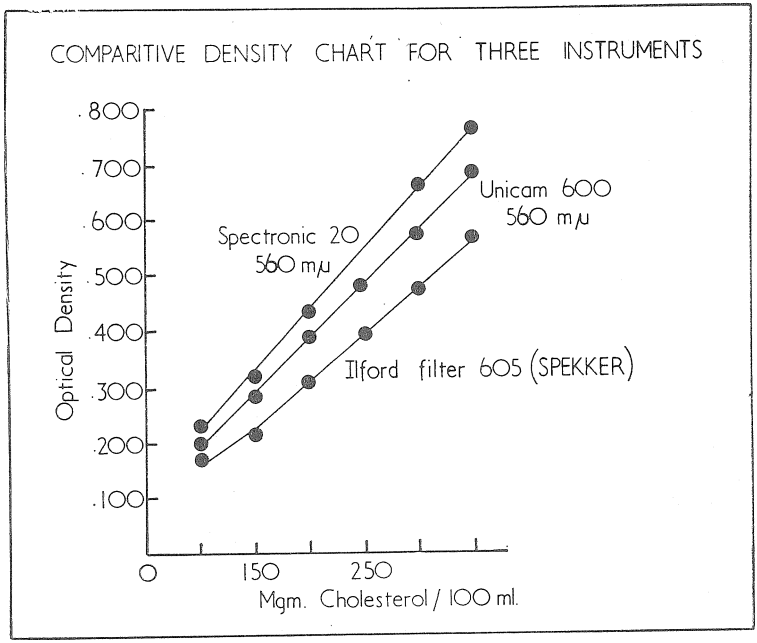


Fig. 5. Calibration of three common laboratory photoelectric instruments.

ml. cholesterol recorded on Unicam 600 at 560mμ, Spectronic 20 at 560mμ and Hilger Spekker (Ilford filter 605) instruments. For the concentrations studied Beer's Law is obeyed. Recovery studies were performed and are illustrated by Table II. In all cases, greater than 95% of added cholesterol is recovered. It has been reported that bilirubin has little, if any, effect on density readings by this method[6] [7]. Evidence may be added by a study on three jaundiced sera of 3.5, 11.6, and 8.0 mg. per 100 ml. bilirubin. After protein precipitation in isopropyl alcohol and centrifugation, clear aliquots of the three sera were again evaluated for bilirubin. The results were 0.1, 0.6 and 0.2 mg. per 100 ml., indicating 97%, 96% and 97% respectively, of bilirubin extracted with the protein precipitate.

As cholesterol extraction depends on shaking of the serum-isopropyl alcohol mixture, tests were carried out to determine variations in total cholesterol when tubes were shaken for 5,

RECOVERY			
Mgms. Cholesterol/100ml. serum			
Initial conc.	Cholesterol added	Final conc.	Percentage recovery
Specimen I 96	50	143	98
	50	144	99
	100	189	97
	100	191	97
Specimen II 182	50	223	97
	50	222	96
	100	270	96
	100	273	97

Table II. Recovery, in duplicate, of two specimens with added cholesterol values of 50 mg./100 ml. and 100 mg./100 ml.

10, 15 and 20 seconds. Results for these times showed no significant variation and subsequently a shaking time of 10 seconds, being physically less variable than a shorter time, was adopted.

The colour reagent, consisting of ferric chloride in phosphoric acid periodically diluted with sulphuric acid, was found to suddenly increase in blank value — probably due to precipitation of ferric ions[10]. This occurred when the final mixture was three weeks old. A modification was adopted in which the ferric chloride, phosphoric acid and sulphuric acid were prepared and mixed directly. This reagent was used for the following studies and after four weeks use showed a stable, low blank value of less than 0.050 optical density.

Proposed Method — Total Cholesterols of Normal Population

Total serum cholesterols were determined on eighty-five apparently healthy, non-hospitalised individuals. Fig. 6 is a histogram showing the distribution of cholesterol levels of the subjects studied. The cholesterol values per age are illustrated by Fig. 7. The mean total serum cholesterol was found to be 208 mg. per 100 ml. and the statistical normal range 140 to 276 mg. per 100 ml.

Finally, by this technique, the standard deviation of twenty-five day-by-day batch control estimations on a serum with a mean value of 178 mg. cholesterol per 100 ml. was 5.6 mg.

Also, a commercially prepared control serum containing added cholesterol of 325 mg. per 100 ml. was reproduced to within 4% accuracy. This standard was also attained by a worker to whom the method was new.

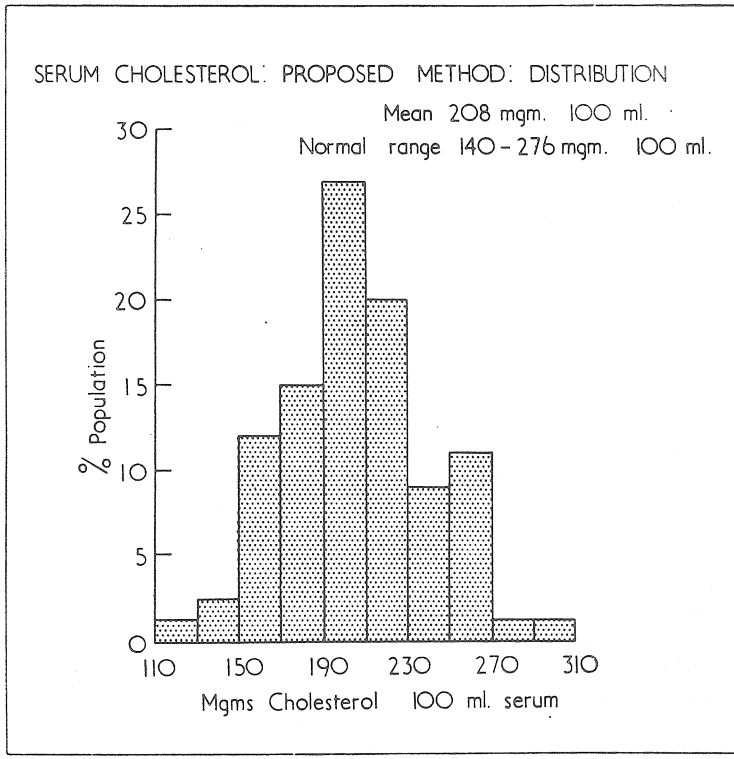


Fig. 6. Distribution of cholesterol values from eighty-five normal subjects by the modified method of Leffler as proposed in this paper.

Summary

The distribution of total serum cholesterol values in a population of one hundred normal individuals was carried out to determine the normal range for an existing method. Figures are given, showing the inordinately high results obtained. An investigation of current techniques, with the object of finding a suitable method comparable with reference techniques, was undertaken. A modification of the method finally selected is described and applied to further studies of a normal population.

Detailed Procedure of Modified Total Serum Cholesterol Method

Principle

Isopropyl alcohol added to serum, produces a finely divided protein precipitate and at the same time extracts cholesterol. A

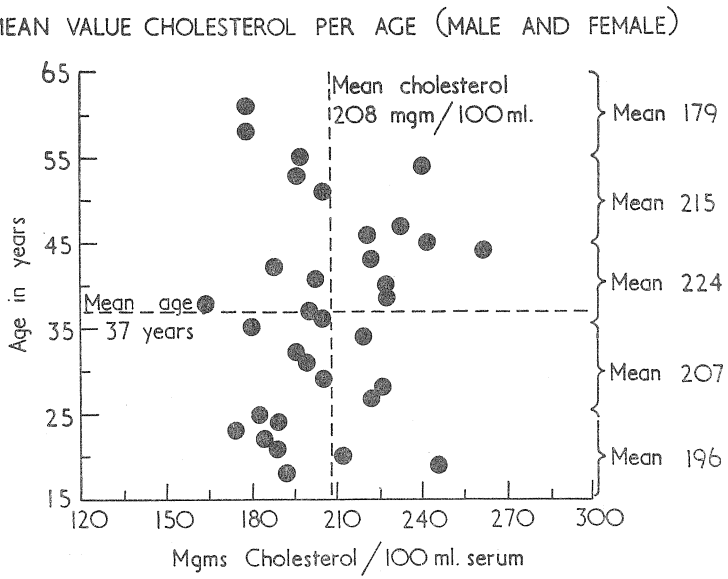


Fig. 7. The same population as in Fig. 6 illustrated to show the distribution by age. Values at the right-hand edge represents the mean for each ten years of age (although for the 55-65 years age-group only two subjects are represented).

protein-free aliquot is taken for colour development with stable ferric chloride colour reagent.

Reagents

1. *Isopropyl alcohol.* Reagent grade.
2. *Iron Solution.* Dissolve 200 mg. ferric chloride ($FeCl_3.6H_20$) in 8 ml. phosphoric acid (H_3PO_4, 87%) and dilute to 100 ml. with concentrated sulphuric acid (H_2SO_4, S.G. 1.84). This reagent should give a negligible blank value and remain stable for four weeks if stored in the dark at room temperature in a glass-stoppered bottle.
3. *Cholesterol stock standard.* 200 mg. of pure cholesterol crystals are dissolved in 100 ml. of isopropyl alcohol. Store in a chemically-clean glass-stoppered bottle at room temperature.
4. *Working standard.* 4 ml. of stock standard are diluted to 100ml. with isopropyl alcohol. One ml. is equivalent to 0.08 mg. of cholesterol.

Procedure

1. 0.1 ml. of serum is pipetted into a clean, dry glass-stoppered tube and 2.4 ml. isopropyl alcohol added slowly with mixing.

2. The tube is shaken vigorously for 10 seconds and left tc stand for three minutes. Centrifuge at 3,000 r.p.m. for 5 minutes.
3. 1 ml. of the clear protein-free supernatant is pipetted into a second glass-stoppered tube. 1 ml. of working standard and 1 ml. of isopropyl alcohol (blank) are added to further tubes and treated similarly.
4. 2 ml. glacial acetic acid are added to all tubes, which are then thoroughly mixed.
5. 2 ml. of colour reagent (iron solution) are pipetted slowly down the side of each tube to form an underlying layer. Tubes are stoppered, inverted six times and left to stand.
6. After fifteen minutes read in a photo-electric colorimeter against the blank at 560mμ or using an Ilford 605 filter and 1 cm. cuvettes. Care should be taken when pouring the final solutions, as the presence of small bubbles will produce false density values.

Calculation

$$\frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 0.08 \times \frac{100}{0.04}$$

$$\frac{\text{O.D. Test}}{\text{O.D. Standard}} \times 200 = \text{mg. cholesterol per 100 ml. serum}$$

Statistical normal range calculation from the determination of 85 normal subjects by this method is 140 to 270 mg. cholesterol per 100 ml. serum.

REFERENCES:

1. Abell, L. L., Levy, B. B. and Kendall, F. E. (1952), *J. biol Chem.*, **195**, 357.
2. Bloor, W. W. (1916), *Ibid.*, **24**, 231.
3. Bowman, R. D. and Wolf, R. C. (1962), *Clin. Chem.*, **8**, 296.
4. Bowman, R. D. and Wolf, R. C. (1962), *Ibid.*, **8**, 302.
5. Burchard (1890), *Chem. Zbl.*, **61**, 25.
6. Leffler, H. H. (1959), *Amer. J. clin. Path.*, **31**, 310.
7. Leffler, H. H. and McDougald, C. H. (1963) *Ibid.*, **39**, 311.
8. Moore, V. R. and Boyle, E. (1963), *Clin. Chem.*, **9**, 156.
9. Rice, E. W. and Grogan, Betty S. (1962), *Ibid.*, **8**, 181.
10. Rosenthal, H. L., Pfluke, M. L. and Buscaglin, S. (1957), *J. Lab. clin. Med.* **50**, 318.
11. Schoenheimer, R. and Sperry, W. M. (1934), *J. biol. Chem.*, **106**, 745.
12. Sperry, W. M. and Webb, M. (1950), *Ibid.*, **187**, 97.
13. Sunderman, F. W. and Sunderman, F. W., Jr. (1960), *Lipids and the Steriod Hormones in Clinical Medicine*, pp 15-22, J. B. Lippincott Co. Philadelphia.
14. Tonks, D. B. (1963), *Clin. Chem.*, **9**, 217.
15. Trinder, P. (1952), *Analyst*, **77**, 323.
16. Zak, B. (1957), *Amer. J. clin. Path.*, **27**, 583.
17. Zlatikus, A., Zak, B. and Boyle, A. J. (1953), *J. Lab. clin, Med.*, **41**, 486.

Macroglobulinaemia — A New Case

M. JEANNETTE GREY

Pathology Department, New Plymouth Hospital.

(Received for publication October, 1963)

Introduction:

Paper electrophoresis and ultracentrifugation have contributed greatly to our knowledge of proteins in human serum. In particular, these techniques led to the discovery of globulins of high molecular weight, the macroglobulins. According to Cantarow and Trumper[1] these are glycolipoproteins representing polymers or other aggregations of smaller globulin molecules. The macroglobulins have a molecular weight of about 1,000,000 as compared to 150,000 for normal globulins. They are believed to originate in lymphoid reticular cells. In 1962 Zucker-Franklin *et al.*[12] showed that lymphoblasts and large and medium-sized lymphocytes were mainly responsible for the synthesis of macroglobulins of the 19S gamma globulin type and that plasma cells and mature lymphocytes were free of these proteins. (19 Svedberg units *i.e.* 19S, is a measure of molecular size as determined by the sedimentation constant in the ultracentrifuge).

In 1944, Prof. J. Waldenstrom[11] of Sweden published an account of three cases of a disease which he later called macroglobulinaemia because it was characterised by grossly increased circulating macroglobulins. Twelve years later, Mackay[4] published the first recorded case of macroglobulinaemia in Australasia. The condition, though fairly rare, seems to occur in most countries; about 80% of cases are over fifty years of age and the majority are males. The course of the disease is variable, though ultimately fatal. However, low grade increases in macroglobulins do occur in some other diseases such as nephrosis, liver disease, congenital syphilis and lupus erythematosis. Macroglobulins are thought to cause surface damage to circulating blood cells and also to vascular endothelium.

Case History Outline:

A. D. G. was a retired farmer of 63 years when he sought medical advice late in 1957 after almost twelve months of intermittent giddiness, lassitude, blurred vision and severe epistaxis. The aggravation of these symptoms by severe influenza caused him to seek treatment and he was admitted to hospital. Prior to the influenza he had been sufficiently well to do casual work such as fencing on the farm and his earlier history appeared to be unconnected with his condition. A.D.G. looked

younger than his years but had lost weight to 9½ stone. He had a normal cardio-vascular system, normal lungs and no deformity or tenderness of bones. Blood pressure was normal and the optic fundi showed none of the retinal haemorrhages that often occur in macroglobulinaemia, though the right fundus was not clearly seen due to apparent old corneal opacity.

X-ray examinations of pelvis, hip, lumbar spine, skull and chest at no stage showed evidence of any destructive lesions to support the original tentative diagnosis of multiple myeloma. Three years later A. D. G. was again in hospital and also five months later, mainly for blood transfusions to treat anaemia due, partially, to severe epistaxes. Eighteen months later he was admitted for excision of a large squamous cell carcinoma of the ear and a smaller early squamous cell carcinoma of the cheek, and for further blood transfusion which by then had totalled more than twenty units since 1957. At this stage A. D. G. was well enough to take a trip overseas. In 1963, owing to the slow course of the disease, the diagnosis of multiple myeloma was reviewed. A revised diagnosis of macroglobulinaemia emerged finally on the facts from ultracentrifugation, prompted by a positive Sia's test suggesting macroglobulins in his serum. A. D. G. died a short time later.

Bone Marrow and Peripheral Blood:

According to Mackay[5], classically the bone marrow is rather densely infiltrated with lymphocytic cells, often with scanty or absent cytoplasm, and sometimes a plasma cell increase occurs. In 1957 A. D. G.'s marrow contained abnormally large numbers of atypical lymphoid cells and plasma cells together with a number of tissue mast cells. Waldenstrom[11] states that this abnormal proliferation of tissue mast cells may often occur in macroglobulinaemia. A. D. G.'s marrow report of six years later stated that the principal cells were still mononuclear forms resembling large lymphocytes but generally with abundant bluish cytoplasm and fairly large round nuclei with skein-like chromatin.

The peripheral blood of A. D. G. showed most of the characteristics described in cases of macroglobulinaemia. His E.S.R. was increased. He had a chronic and slowly progressive anaemia and the gross rouleaux formation in his blood was typical. In 1957, prior to any transfusion, his haemoglobin was 7 grams% and his P.C.V. 23%. His leucocyte counts were consistently at the lower limits of normal (4-6,000 per cmm.) and his original differential picture showed 54% lymphocytes, some of which were atypical forms. However, during the seven year illness his neutrophil/lymphocyte ratio was fairly normal. Blood films were often poor due to rouleaux and increased blood viscosity. The platelets were greatly reduced in numbers and by

1962 the figure was only 36,000 per cmm., falling to 11,600 per cmm. shortly before his death. A. D. G. had prolonged bleeding times and in macroglobulinaemia this haemorrhagic tendency is due to complex disturbances in the clotting mechanism as reviewed by Mackay[5]. These include interference with platelet function by macroglobulins.

Proteins and Electrophoresis:

The total protein is usually high in macroglobulinaemia and A. D. G.'s was no exception: in 1957 it was 11.1g./% rising to 12.5g./% (1963). The albumin is usually decreased, with a reversed albumin/globulin ratio. A. D. G.'s albumin was 4.1g./% (1957) and 4.3g./% (1963) with a globulin of 7g./% (1957) rising to 8.2g./% (1963).

In macroglobulinaemia, normal gamma globulin is at a low level with resulting immunological failure. The electrophoretic pattern resembles that of multiple myeloma with a markedly increased band in the gamma globulin area, heavy but fairly discrete. In 1957 A. D. G.'s serum followed this pattern but by 1963 the amount of macroglobulin migrating with the gamma globulin was so great that a much more diffuse band occurred. The decreased albumin was obvious at all times but the alpha and beta globulin fractions were apparently normal at first. By 1963, however, a decrease was apparent in these bands. Pachter[8] states that there is often a high content of carbohydrates in macroglobulinaemia, evidenced by Periodic Acid Schiff staining of electrophoretic paper strips. Unfortunately this was not tested.

No cryoglobulins were detected in A. D. G.'s serum, though often macroglobulins become so viscid with a fall in temperature below 20°C. that they form a gel and so resemble one type of cryoglobulin[5]. Bence-Jones protein was not detected in A. D. G.'s urine at any stage of the illness; it is reported as 'not infrequent' in macroglobulinaemia[5].

A. D. G.'s thymol turbidity of 13 Maclagan units with a thymol flocculation of 4+ is interesting as Martin[6] describes 18 cases of macroglobulinaemia, all of which gave a positive thymol turbidity in contrast with 14 cases of myelomatosis of which only one gave a positive thymol turbidity. Kunkel's gamma-globulin precipitation in buffered zinc sulphate was so dense with A. D. G.'s serum that an accurate figure was not obtainable.

In 1963, other blood chemistry of A. D. G. gave normal serum acid and alkaline phosphatases, a plasma cholesterol of 132 mg./100 ml. and a blood T.N.P.N. of 60 mg./100 ml. The original T.N.P.N. in 1957 was 55 mg./100 ml. Post-mortem examination showed some kidney damage.

The viscosity of A. D. G.'s serum in his last few months

was very high even at room temperature, and intense centrifugation was necessary to free the serum from erythrocytes.

Sia's Test:

In the early years of A. D. G.'s case, Sia's test was overlooked. It was this extraordinarily simple test that led to the diagnosis of macroglobulinaemia which was confirmed by ultracentrifugation.

Sia's test was originally devised thirty years ago in connection with the diagnosis of Kala-azar. It consists basically in letting a drop of the patient's serum fall into distilled water. In a positive reaction the drop of serum becomes a heavy white precipitate but normal serum gives only a greyish haze which rapidly disperses[11]. A. D. G.'s serum gave an immediate and very heavy precipitate.

According to Martin[6], sera with 8% or more of globulins of 17 Svedberg units (or greater) diluted 1:20, form a dense cloud and sediment within 5 minutes or less when the molar concentration of electrolytes is reduced to 0.01 or lower. Thus, a further modification of the test is the separation of the precipitate followed by washing with distilled water and redissolving in 0.15M saline. The control of the initial pH to 6.5-7 and temperature to 20°C. is advisable. This modification avoids false positives and confusion with weaker Sia-positive sera arising from Leishmaniasis or myelomatosis.

Ultracentrifugation:

In 1945, Pedersen of Sweden showed that the discrete but heavy bands in some gamma-globulin fractions in serum electrophoresis contained a dominant component of high molecular weight and by ultracentrifugation, revealed that the sedimentation constant of this fraction is usually 17-19 Svedberg units: it was named macroglobulin or the 20S component. Waldenstrom[11] states that only globulins with a sedimentation constant greater than 15 Svedberg units should be regarded as true macroglobulins. This is the notable feature preventing confusion with multiple myeloma which may give values up to 13 Svedberg units. Minor components of high sedimentation value are often seen near the main macroglobulin peak in macroglobulinaemia[6].

Normal gamma globulin has a value of about 7 Svedberg units. Normal serum may contain up to 5% of 20S component which probably represents antibodies, and the abnormal macroglobulins appear to be unrelated immunologically to normal macroglobulins.

Ultracentrifugation techniques are complex and details may be found in excellent articles by Pickels[9] and by Gray[2]. The equipment is beyond the financial resources of most medical laboratories and I am indebted to Mr J. W. Lyttleton[3], senior

chemist at the D.S.I.R. plant chemistry division, Palmerston North, for the following report of A. D. G.'s serum in March 1963:—

> " The analyses of the A. D. G. serum were made on a Beckman Model E Analytical Ultracentrifuge. The first series of photographs were taken at eight minute intervals during a run at 59,780 r.p.m. on a sample of serum diluted 5-fold with physiological saline solution; the second series were taken at 52,640 r.p.m. at 0, 16, 32, 40 and 48 minutes. In this run two concentrations were studied, 10-fold and 20-fold dilutions with saline.
>
> " The sedimentation coefficient of the macroglobulin at the 5, 10 and 20-fold dilutions was 12.2, 14.9 and 15.9S. This extrapolates to a value of 17.8S at infinite dilution. This figure alone does not give you the molecular weight, other than indicating that it is a large molecule probably greater than 500,000, but it may enable you to compare it with other published values. The macroglobulin accounted for 58% of the total serum protein, and in the original serum its concentration was 7.3 g./100ml."

McFarlane *et al.*[7] write of a macroglobulin with a sedimentation coefficient of 18, having a molecular weight of 1,160,000. It would therefore seem likely that A. D. G.'s macroglobulin molecular weight was greater than 500,000 and probably near 1,000,000.

Recent work on physico-chemical and immunological aspects of pathological macroglobulins by Ratcliff *et al.*[10] (1962) with a gel-diffusion precipitin technique using a specific antiserum to 19S components of gamma globulin, has shown this method as a possible substitute for the ultracentrifuge in the identification of pathological macroglobulins. Ratcliff *et al.*[10] also gives data relevant to the above report on ultracentrifugation.

Summary:

A new case of macroglobulinaemia is reviewed in parallel with accepted concepts of the disease, with emphasis on its diagnosis by laboratory methods including confirmation by ultracentrifugation.

Acknowledgments:

My thanks are due to Dr D. N. Allen and to Dr M. C. Chapman for permission to use the laboratory reports and the case notes, to Dr A. C. Hayton for helpful criticism of this paper and to Mr J. W. Lyttleton (D.S.I.R.) for the report on the serum ultracentrifugation.

REFERENCES:

1. Cantarow, A. and Trumper, M. *Clinical Biochemistry,* 6th Ed., p159, W. B. Saunders and Co., Philadelphia.
2. Gray, G. W. (1951), *Sci. Amer.* **185,** 43.
3. Lyttleton, J. W. (1963), Personal communication.
4. Mackay, I. R. (1956), *Aust. Ann. Med.,* **5,** 244.
5. Mackay, I. R. (1959), *Ibid,* **8,** 158.
6. Martin, N. H. (1960), *Quart. J. Med.,* **29,** 179.
7. McFarlane, A. S., Dovey, A., Slack, H. G. B., Papastamatis, S. C. (1952), *J. Path. Bact.,* **64,** 335.
8. Pachter, M. R. (1959), *Postgrad. Med.,* **26,** 815.
9. Pickels, E. G., *Methods in Medical Research* (1953) Vol. V, ed Corcoran, A. C., Year Book Publishers Inc., Chicago.
10. Ratcliff, P., Soothill, J. F., Stanworth, D. R. (1963), *Clin. chim. Acta.,* **8,** 91.
11. Waldenstrom, J., (1958), *Triangle,* **3,** 262.
12. Zucker-Franklin Dorothea, Franklin, E. C., Cooper, N. S. (1962), *Blood,* **20,** 56.

The Junior Essay Competition

In accordance with the terms of Rule 27 of the New Zealand Institute of Medical Laboratory Technology (Inc.), a prize of £5 5s 0d is awarded annually for the best entry in each of the two sections of the Junior Essay Competition.

TECHNICAL SECTION: Consisting of descriptions of methods or technical procedures. Entries in this section should be presented in the manner laid down in the 'Directions for Contributors' appearing on the inside front cover of some issues of the *Journal.*

ESSAY SECTION: Consisting of essays on general, historical or particular aspects of medical laboratory technology, and presented in the style of *an essay.* The directions regarding double spacing, nomenclature and references are also applicable in this section, but it is expected that entries will take the form of a continuous literary composition, 1,000 to 1,500 words in length and unbroken by sub-headings.

All trainees are eligible to enter.

Entrants are advised that they should indicate for which section of the Competition they wish to enter, and should give their name and address on a separate piece of paper.

Due regard will be paid in the judging to the mode of presentation, and the Council of the Institute reserves the right to withhold the awards in any year when no entry reaches a desirable standard.

Entries should be submitted to the editor of this journal to reach him not later than May 29, 1964.

A Comparison of Methods for the Estimation of the Erythrocyte Sedimentation Rate

JILLIAN E. MONTGOMERIE
Pathology Department, Palmerston North Hospital.

(Received for publication May, 1963)

One of the oldest investigations in clinical medicine, the Erythrocyte Sedimentation Rate (E.S.R.) seems to have developed from the observations of the ancient Greeks. They realised that the separation of blood into four layers — the four humours — was the result of rather than the cause of disease. The E.S.R. is now used as a non-specific screen for detecting a somatic response to an organic disease, and to assess the progress of the illness.

A variety of methods have been used to estimate the E.S.R. Some, for example that of Linzmeir, record the time for the erythrocytes to reach a focal point; others, such as those of Wintrobe and Westergren, note the depth of the plasma column after the blood has stood for a fixed time. Alternatively, several readings may be made at stated intervals and a curve plotted; the steepness of the curve indicating the severity of the disease.

Recently, interest has been revived by Gilmour and Sykes[2] and Dawson[1], who showed the divergence in results obtained by the two standard methods now in use.

A series of comparisons was made, since local findings also showed this discrepancy.

The aims of the study were:—

1. (a) To compare the E.S.R.'s estimated by Wintrobe's method with those obtained by Westergren's method.
   (b) If there was a difference, to find which result most closely corresponded with the clinical state of the patient.
2. If the Westergren method proved more reliable, to see if the results obtained by the Classical method were comparable with a modification[3]. In this modification the blood was collected over a dry anticoagulant and then diluted 1 in 5 with 3.8% trisodium citrate as for the classical method.

Material and Methods.

The patients were selected because, although their clinical notes indicated that a raised E.S.R. would be expected, the rate estimated routinely by the Wintrobe method showed normal or slightly raised values. A number of other patients were chosen because although their blood films showed marked rouleaux formation, they had a normal or only a slightly increased Wintrobe Sedimentation Rate.

The blood was obtained, with minimal trauma, from the anticubital vein after cleansing the skin with antiseptic solution and allowing it to dry. The blood was obtained for both methods at the same time. After removing the needle from the syringe, the blood was added to tubes containing the appropriate anti-coagulant. Immediately the tubes were inverted ten times tc ensure thorough mixing, and on arrival in the laboratory, thc samples were placed on a mechanical mixer (33 1-3 r.p.m. turntable) for three minutes before testing.

The blood samples were then tested in the following ways:—

1. *Method of Wintrobe.*
   An haematocrit tube 110 mm. long with a bore of 2.0-2.5 mm. was used.
   1.5 mg./ml of Sequestrene (di-potassium salt of Ethylene diamine tetra-acetic acid - E.D.T.A.) was used as the anti-coagulant of choice for the following reasons.
   (a) It is in routine use at this laboratory.
   (b) Sequestrene is said to shorten the first phase in the sedimentation phenomenon (see later) and to reduce the tendency of the cells to shrink, thus increasing the accuracy of the E.S.R.
   (c) The di-potassium salt was chosen in preference to the di-sodium salt because it is more water soluble. The respective solubilities being 20% w/v and 6% w/v.
   After thorough mixing, the Wintrobe tube was filled with blood to the 100 mm. mark, placed in a vertical position and left undisturbed for one hour exactly.
   The normal range for this method was taken to be
   1 - 20 mm. in one hour for women.
   0 - 9 mm. in one hour for men.
2. *Classical method of Westergren.*
   A tube closely resembling a 1 ml. pipette, 200 mm. in length and 2.5 mm. in diameter.
   After mixing, the blood was diluted 1 in 5 with 3.8% aqueous trisodium citrate. The blood was drawn to slightly above the 200 mm. mark and allowed to reflux back to the mark. After clamping the tubes into a special rack, they are left undisturbed for exactly one hour.
3. *Modified method of Westergren.* (Goldbergh and Conway)[3]
   After withdrawal the blood sample was delivered into a tube containing 1.5 mg./ml. Sequestrene. It was diluted later after mixing 1 in 5 with 3.8% trisodium citrate and treated as for the classical Westergren method.
   The normal range for both the Westergren methods was taken to be
   4 - 7 mm. in one hour for women.
   3 - 5 mm. in one hour for men.

The same timer was used for all the tests and the E.S.R. was read as:—

The distance, measured in mm., from the bottom of the surface meniscus to the top of the erythrocyte layer on the upper surface of the buffy coat. In cases where the red cells settle in a diffuse rather than a compact layer, the reading was taken at the most compact upper layer of the erythrocyte column.

Results.

1. Comparison of the Wintrobe and the classical Westergren methods. 100 estimations were made.
2. Comparison of the classical and modified Westergren methods. 50 estimations were made.
   See figures 1 and 2.

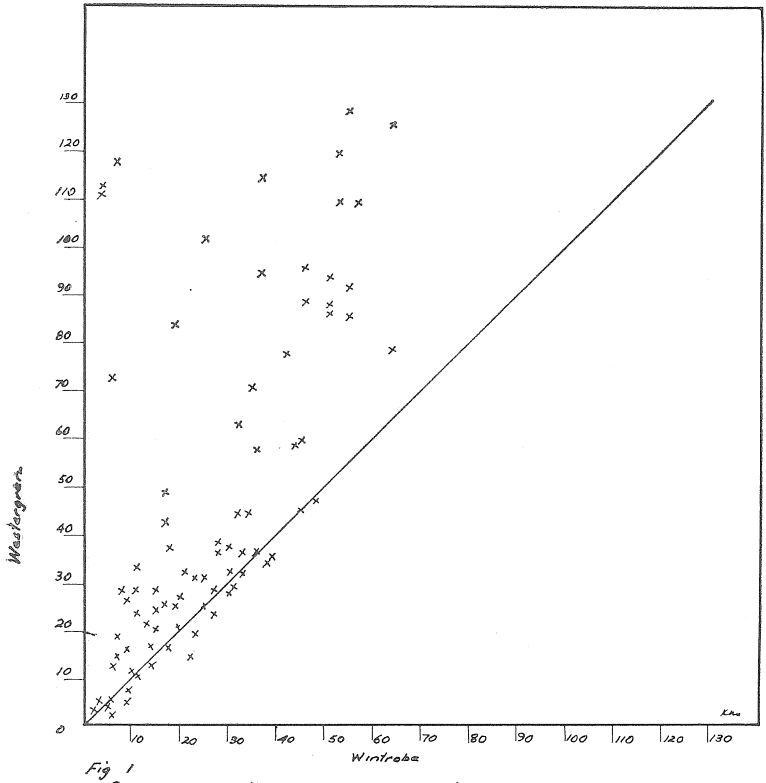


Fig 1
Comparison of the Wintrobe and classical Westergren methods
THE FIXED LINE REPRESENTS 45° LINE ie. The line upon which the points should fall, if the methods on the two axes give identical results.

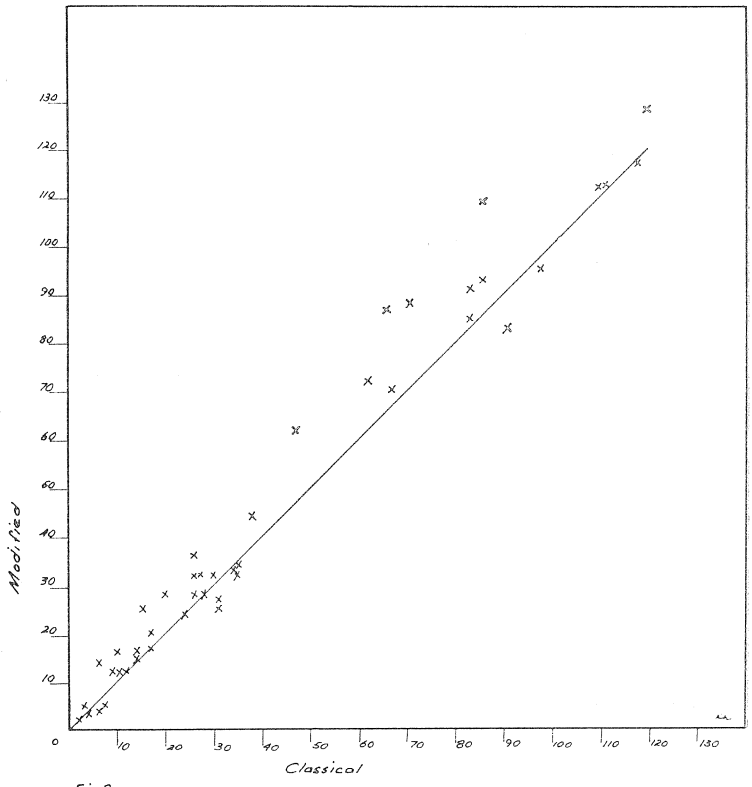


Fig 2.
Comparison of the classical and modified Wastergren methods.

Discussion:

Dawson[1] states that it is now generally believed that an accurate and reproducible E.S.R., if based on known normal standards, does, if raised, indicate a non-specific somatic response to disease. The more important of these physico-chemical factors which may effect the E.S.R. are set out below.

1. The column should be as high as possible as the cells may pack prematurely in a short column and therefore give misleading results.
2. The internal bore of the tube should be more than 2.5 mm. to avoid capillary action, which may also have a retarding effect on the sedimentation rate.
3. Controls such as a plumb line or spirit level should be fitted to every rack. When tubes vary from the vertical, they do not meet the full resistance of displacement and an error is introduced.

4. The choice of a solid (sequestrene) or a liquid citrate anticoagulant and the care in mixing also effects the E.S.R. A dilution factor is introduced with the use of a liquid anticoagulant, which also lowers the plasma viscosity.
5. Trauma, cleanliness and contamination with antiseptics are all factors affecting the sedimentation rate.
6. Time, which may be divided into
   (a) Total time over which the estimation is made.
   (b) The time between the taking of the specimens and the estimation of the sedimentation rate. A progressively larger reduction in the rate of sedimentation occurs after four hours, due to the ageing of the erythrocytes, which lose their susceptibility to clumping and rouleaux formation.
7. Temperature: the optimum is 22-27°C. No effect is found over the first eight hours storage at 15-23°C, but beyond this time a rise in temperature, in particular, increases the errors associated with storage.
8. Personal factors such as frustration, clumsiness and fatigue may all have an effect on the results of the test.

It has been shown that when anticoagulated blood is subjected to standard physico-chemical variants in a suitable tube it undergoes three major phases. (Fig. 3)

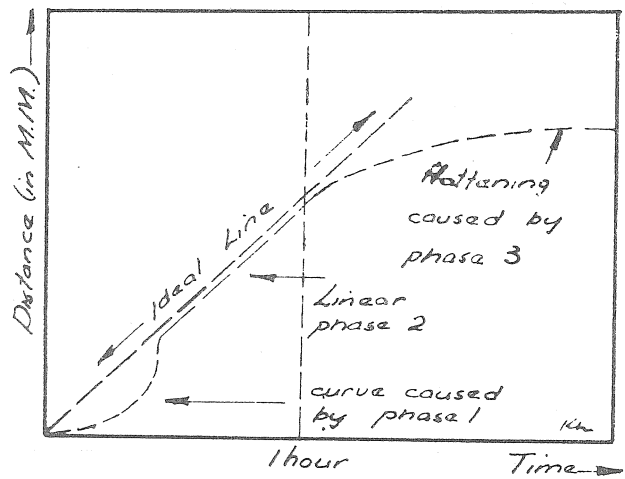


Fig 3

The Three Phases of an E.S.R. Estimation

1. The formation of rouleaux, which should be as short as possible as this is included in the time measured.
2. The fall under steady gravitational influence; ideally this is the only phase measured.
3. The flattening of the curve due to packing.

From the results in the graphs it can be seen that there is a divergence in the results obtained by the Westergren and Wintrobe methods. From collateral evidence it would seem that the Westergren technique depicts more accurately the clinical state of the patient. In a number of cases the Wintrobe E.S.R., routinely estimated, gave normal results when the clinical picture was suggestive of an organic disease. The Westergren E.S.R. was found to reflect this. (Table I)

Several factors are thought to contribute to the unreliability of the Wintrobe method, some of which are listed below.

1. The type of anticoagulant used. No dilution factor is introduced by this technique, since the anticoagulant used is in a solid form. Anomalous results occur with undiluted blood when the blood shows a high plasma viscosity as well as a high haematocrit. A high plasma viscosity retards the downward movement of the cells and the corresponding upward movement of the plasma. The dilution of the blood therefore lowers the plasma viscosity, allowing the more rapid sedimentation of the erythrocytes in the presence of abnormal proteins. The dilution factor seems to be of some importance, as the E.S.R. reflects the degree of disturbance of the protein fractions.

   A number of patients showing marked rouleaux formation in their blood films, but with normal sedimentation rates by Wintrobe's technique, showed markedly raised E.S.R.'s by the Westergren methods.

Wintrobe	Westergren	
	Modified	Classical
4	111	117
4	110	112
7	118	117
6	62	72
8	26	28
11	33	34

TABLE I

2. The bore of the tube, which seems to vary according to the make, and may be between 2.0 and 2.5 mm. in diameter. It has been stated that approximately parallel results can be obtained by the Wintrobe and Westergren methods, when the Wintrobe test is performed in tubes with a bore of

4-5 mm. The wider bore lowers the frictional resistance between the plasma and the wall of the tube in undiluted bloods of high viscosity.

3. The length of the Wintrobe tube is only 100 mm. compared to the Westergren tube which is 200 mm. It is therefore impossible to obtain an E.S.R. of greater than 60-70 mm. (depending on the haematocrit) due to the packing of the red cells. The longer tube, for the same reason, lengthens the time of the second period *i.e.* rouleaux formation with constant fall, which is ideally the only period timed.

On the other hand, the Wintrobe technique has some obvious advantages, some of which are only theoretical in application.

1. The test is simpler and less time consuming to carry out as no dilution needs to take place.
2. The popularity of the Wintrobe technique is due mainly to the fact that an estimation of the haematocrit can be made following the E.S.R. The estimation of the haematocrit also enables the sedimentation rate to be corrected for anaemia by means of specially composed charts. These corrections have been thoroughly discredited in literature and are said to be crude and artificial. Sometimes, when anaemia is severe, the corrected rates are misleading as negative values are obtained.

However, with the introduction of micro-haematocrit the advantage of the packed cell volume following the E.S.R. has been removed.

Although the classical and modified Westergren methods show a slight diversity in results, it is thought that in practice this variation is not of clinical importance, since they both show normal and raised results in parallel. The variation seems to be more marked as the E.S.R. increases. The divergence may be due in part to technical error and to inaccuracies in the equipment. For example, much difficulty was encountered in measuring blood accurately from a syringe.

Although the modified Westergren technique would appear to be more laborious than the classical method, this appears to be outweighed by the very convenience of being able to estimate E.S.R.'s on the same sample of blood as is used for haematological screening, which, of course, must be received undiluted.

Summary:

The E.S.R. is merely a simple, rapid and fairly crude method by which pathological changes in the plasma proteins may be revealed. It therefore seems apparent, as the E.S.R. is used to assess the activity of a disease, that it is essential to choose a method which most accurately reflects the disease process. The

Westergren Erythrocyte Sedimentation Rate appears to be the method that most accurately does this.

It would appear that although the results determined by the modified Westergren technique differ slightly from those estimated by the classical method, the variation is of no significance. Some authors claim that results obtained by that method are more reliable and reproducible than those estimated by the classical Westergren method.

REFERENCES:
1. Dawson, J. B. (1960), *Brit. med. J.*, **ii**, 1697.
2. Gilmour, D. and Sykes, A. J. (1951), *Ibid*, **ii**, 1496.
3. Goldbergh, A. and Conway, H. (1952), *Ibid*, **ii**, 315.

Letters to the Editor

PASTEURELLA MULTOCIDA IN SPUTUM

Dear Sir,

I was most interested to read Mr Miller's article on the isolation of *Pasteurella multocida* in the October 1963 *Journal*.

In March of this year we isolated the same organism in almost pure culture from a muco-purulent sputum. The patient, a male aged 35 years, presented with a history of chronic bronchitis with a productive cough. The identification of the organism as *P. multocida* was confirmed by the Communicable Disease Centre Laboratory, Georgia, U.S.A.

This was the first time *P. multocida* had been isolated in this laboratory, but, until reading Mr Miller's article, we were unaware of the comparative infrequency with which this organism is encountered in routine specimens.

RITA E. LOWES.
c/o Drs Taylor and Lycette,
Knight St., Hastings.
October 17, 1963.

EXAMINATIONS

Dear Sir,

I hope that with the introduction of the new examination syllabuses we have heard so much about, we shall see some sort of reason in the kind of questions asked of candidates. One feels that it is hardly fitting for Final entrants to be asked to show their ability in carrying out a full blood count, as in the October 1963 examination for example; and perhaps someone could advise me, for future guidance, in the instruction of my trainees, what is the difference between the tests to be performed on a donor sample at the fifth donation, as distinct from the first or, for that matter, the fifty-first donation.

MENTOR,
December 1, 1963.

[Abridged.—Ed.]

Abstracts from Other Journals

Contributors to this issue: R. D. Allan, J. Case, T. E. Miller, J. Rees, H. C. W. Shott, D. Tingle.

BLOOD BANKING

The Specificity of the Antibody in Paroxysmal Cold Haemoglobinuria. Levine, P., Celano, M.J. and Falkowski, F. (1963), *Transfusion,* **3,** 278.

The antibody in four cases of paroxysmal cold haemoglobinuria was investigated for specificity and shown to be Anti-P + P_1 (Anti-Tj^a).

False Positive Coombs Tests Due to Bacterial Contaminants in Stored Pilot Tube Blood. Beck Munzer, Grace E., Laux, France A., Rowe, R. D. and Przybyla, Mary J. (1963), *Transfusion,* **3,** 283.

An experiment was conducted to explore the incidence of false positive direct and indirect antiglobulin tests on contaminated bloods. Results showed that although no false results were obtained with some organisms, such bacteria as *Pseudomonas effusa* and *Bacillus subtilis* were capable of causing misleading positive reactions.

A Modification of the Anti-Globulin Test with Increased Sensitivity. Hiern, B.C. (1963), *Bull. Tulane med. Fac.* **22,** 231.

In this work, carried out towards an M.D. thesis, the author has shown increased sensitivity of anti-globulin tests using an ingenious technique. The cells to be tested are washed and suitably diluted rabbit anti-human globulin is added in the usual way. If weak or negative results are obtained, the cells are now washed again and sheep anti-rabbit globulin serum is added. The tubes are centrifuged and examined again for agglutination. A striking enhancement of the ability to detect antibodies coating the erythrocytes is claimed.

Erythroblastosis Due to Anti-Rh 6. Freda, F. J., D'Esopo, A. D., Rosenfield, R. E. and Haber, Gladys V. (1963), *Transfusion,* **3,** 281.

This paper describes a case of haemolytic disease of the newborn which is attributed to the rare rhesus antibody anti-f. Only a few examples of this antibody, first reported in 1953, are to be found in the literature—and there are no previously recorded cases of haemolytic disease in which it is the only detectable antibody. The antibody reacts with cells representing the genes c and e in the *cis* position and, when present, is generally in persons of the phenotype CcDE (probable genotype CDe/cDE).

In the case described, the antibody was detected in the maternal serum during ante-natal screening, and its titre was 64 by the indirect antiglobulin technique. The infant had a positive direct antiglobulin test and a cord blood bilirubin level of 0.4 mg. per 100 ml., which rose to 21.7 mg. per 100 ml. at 82 hours and necessitated exchange transfusion. Anti-f was eluted from the infant's cells, which were f positive, and the antibody was detectable in the infant's serum.

Hemorrhagic and Hemolytic Transfusion Reaction Due to Anti-Le^a. MacPherson, C.R., Teteris, N.T. and Claasen, L.G. (1963), *Transfusion,* **3,** 392.

The two lessons to be learnt from this case report are that antibodies which are normally of the cold type may occasionally develop immune characteristics, and that for safety during multiple transfusions, the crossmatching tests need to be carried out on fresh specimens of the patient's blood taken at suitable intervals.

The patient whose case is described suffered a severe haemolytic and haemorrhagic transfusion reaction due to the antibody Anti-Le^a, which was of the immune type and developed during the course of a series of transfusions.

Copper Sulfate Screening of Blood Donors. Mannarino, A.D. and MacPherson, C.R. (1963), *Transfusion,* **3,** 398.

This short paper calls attention to the fallibility of the copper

sulphate screening test in patients with hyperproteinaemia. The case cited is of a woman with multiple myeloma, who was passed as suitable to donate blood when her haemoglobin level was less than 8 g. per 100 ml.

Elution of Antibodies from Sensitized Red Blood Cells by Means of Cesium Chloride. Anderson, R.E. and Walford, R.L. (1963), *Amer. J. clin. Path.*, **40,** 325.

A technique is described for the elution of antibodies from red cells using cesium chloride as an eluting agent. Sensitized red cells are treated with CsCl solution and the supernatant dialyzed overnight in 0.9% sodium chloride, then the volume is readjusted with 0.9% sodium chloride. The authors found this method superior to NaCl in dissociating antigen/antibody complexes at a variety of molarities. J.R.

The Identification of Anti-I Agglutinins in Human Serum. Jakobowicz, Rachel and Simmons, R. T. (1964), *Med. J. Aust.*, **i,** 194.

This paper describes the discovery, in the serum of a pregnant woman of Greek descent, of a cold-reacting complete antibody, and its progressive identification as anti-I. The antibody proved to be non-placenta permeable, and the infant later born was not suffering from haemolytic disease.

CHEMICAL PATHOLOGY

The Value of the Urease-Strip Test for Estimating Blood Urea Concentration. Grabener, E. (1963), *Germ. med. Mth.*, **8,** 419.

A series of 103 randoms and 85 bloods of known elevated blood urea levels was examined. Duplicate determinations were carried out using the rapid chromatographic urease screening test and a photometric urease method, and the former was found to give reliable and reproducible results when the blood urea level was less than 90 mg/100ml.

Serum Calcium and Magnesium Estimation. Beale, B. and Bastrow, J. (1963), *J. clin. Path.*, **16,** 252.

E.E.L. Titrator and Corinth B indicator.

Confidence limits Ca $\pm$ 1.5% Mg $\pm$ 1.8%.

Sufficiently sensitive to obviate estrapolation of slopes. R.D.A.

Thin Layer Chromatography for Amino Acids and Sugar. Baron, D. N. and Economidis, J. (1963), *J. clin. Path.*, **16,** 484.

Simple methods are described, including a simple way of preparing plates without expensive apparatus. R.D.A.

Stability of Glucose in Serum. Ruiter, J., Weinberg, F., and Morrison, A. (1963), *Clin. Chem.*, **9,** 356.

The destruction of glucose does not occur in separated serum during 48 hours following collection. Blood was allowed to clot and separated up to two hours after. A profound glycolysis occurred in specimens left 24 hours before separating. R.D.A.

Phosphoglucose Isomerase. Horrocks, J. E., Ward, J., and King, J. (1963), *J. clin. Path.*, **16,** 248.

Simple colorimetric method. Substrate is disodium-glucose-6-phosphate. Colour reagent is resorcinol thio-urea. A 75°C. water bath is required. Useful for following palliative treatment for Ca breast or prostate. R.D.A.

Serum Calcium Estimation. Malinstadt, J. and Hadjiioannou, F. P. (1963), *Clin. Chem.*, **9,** 423.

Calcium auto titration with Calcon and a 'spectro electro' unit and constant rate motor-driven burette. Straight glass stirrer and underliquid delivery.

1. Mg is added to standard as it retards end point.
2. Abnormally high Ca and PO_4 cause premature end-point or no automatic termination at this point, due to precipitated calcium phosphate at the high pH (13). In such cases use less serum.
3. Precision increased by adding 0.1 ml. Std Calcium to 0.1ml. serum.
4. Add a drop of KCN to mask Fe and Cu if serum haemolysed.

R.D.A.

Automation of Protein Bound Iodine. Steven, C. O. and Levandoski, N. C. (1963), *Clin. Chem.*, **9**, 400.

Automation of P.B.I. The colorimetric part of the Barker alkali-incineration method can be automated, avoiding the successive readings of the manual method. Good agreement. R.D.A.

Ibid 9, 408, Benatti *et al.* describe a similar technique for P.B.I. and Butanol extractable iodine (B.E.I.). R.D.A.

The Reliability of the Albustix Test for Proteinuria. Wills, M. R., and McGarvan, G. K. (1963), *J. clin. Path.*, **16**, 487.

Trace false positives with fresh urine may be due to mucus. Strongly alkaline urines may give strong false positives unless acidified. Certain detergents are also suspect. Two cases of false positives are cited, which could be attributed to any of the above reasons. R.D.A.

CYTOLOGY

A Routine Method for Staining Eosinophils in Sputum. Payne, R.W. and Hill, R.H. (1963), *J. clin. Path.*, **16**, 387.

Carbol-chromatrope 2R is used as a counterstain with haematoxylin, and is found to give good reproducible appearance of the eosinophils, which are easier to interpret than those seen in preparations stained with haematoxylin and eosin. J.R.

HAEMATOLOGY

Use of a Prothrombin Meter for Quick's One-Stage Test. Jacobs, A. G. and Freer, J. A. (1963), *Brit. med. J.*, **ii**, 978.

A comparison of prothrombin times estimated manually and those determined by a photo-electric method is described and discussed.

There seems to be a tendency for the machine to overestimate times in excess of 30 seconds, but there is tolerable accuracy when prothrombin times are within the therapeutic range.

The Origin of 'Burr' Erythrocytes. Bell, R. E. (1963), *Brit. J. Haemat.*, **9**, 552.

The possible mechanisms by which 'burr' red cells are produced in the blood of patients with uraemia, certain haemolytic anaemias and in other conditions, is explained and discussed.

Rapid Measurement of Hemoglobin A_2 by Means of Cellulose Acetate Membrane Electrophoresis. Rozman, R. S., Sacks, R. P. and Kates, R. (1963), *J. Lab. clin. Med.*, **62**, 692.

Using this method, a statistically significant difference was obtained between normal bloods and those from patients with thalassaemia minor. Values between 2.4 and 4.1% of haemoglobin A_2 were found in the group of normals, while those obtained in the group of sufferers from thalassaemia minor fell between 5.4 and 7.8%.

Quantitative Assay of Fibrinogen and Fibrinolytic Activity. Perkins H. A. and Rolfs, Mary (1963), *Blood*, **22**, 485.

Fibrinogen assays were performed by a slightly modified Ratnoff-Menzie technique. Fibrinolytic activity was estimated by duplicate assays employing four-hour incubation of the diluted clot, lysis being inhibited in one of the tubes by epsilon-amino-caproic acid.

The technique showed significant degrees of fibrinolytic activity in the plasma of most patients after open-heart surgery as compared with the normal controls. J.R.

A Rapid Micro-Method for Recording Red Cell Osmotic Fragility by Continuous Decrease of Salt Concentration. Danon, A. (1963), *J. clin. Path.*, **16**, 377.

A very small volume of a 1 in 10 dilution of whole blood in isotonic sodium chloride solution is introduced into a container, two walls of which are made of a dialysing membrane. The container is placed in a test tube of distilled water, which is in turn placed into a colorimeter

which is equipped with a recorder. Dialysis through the membrane results in a continuous decrease in the salt concentration of the medium surrounding the erythrocytes. The increasing transparency of the erythrocyte suspension while haemolysis takes place is the measurement of the degree of haemolysis. Recording the increasing light transmission as a function of time (*i.e.* as a function of decreasing salt concentration) gives the osmotic fragility curve.

This automatically-recorded curve is obtained in less than ten minutes. J.R.

Counting Platelets with the Aid of an Electronic Counter. D'Angelo, G. and Lacombe, M. (1963), *Canad. J. med. Technol.*, **25**,182.

During a period of thirty months, platelet counts were carried out on a Coulter electronic counter using a technique involving di-potassium E.D.T.A. anticoagulant, ' Lusteroid ' tubes and a brief period of centrifuging to separate platelets from other cellular elements. Comparison with a haemocytometer method and control by examination of stained films showed the method to be reliable and reproducible.

The Coagulation Time. Horsnell, Lillian D. (1963), *Canad. J. med. Technol.*, **25**,186.

Three methods of determining coagulation time are compared, and the Lee & White method is commended as being the most reliable, chiefly owing to the freedom from contamination with tissue juices and the sharpness of the end-point.

HISTOPATHOLOGY

A Paraffin Embedding Technique for Studies Employing Immunofluorescence. Sainte-Marie, G., (1962), *J. Histochem. Cytochem.* **10**,250.

Fresh tissues are fixed in cold 95% ethanol at 4°C. Dehydration and clearing in absolute alcohol and xylene are carried out at the same temperature. Embedding in paraffin wax is performed in the usual manner and blocks should be cut immediately or stored at 4°C. When cutting the sections, contact with water is kept to a minimum. After drying at 37°C for ½ hour, the sections are taken through cold xylene and alcohol and washed in cold buffer solution prior to exposing to the appropriate immunological reagent.

The method shows more precise localisation of antibody or antigen than can be obtained using frozen sections. D. T.

Studies on the Character and Staining of Fibrin. Lendrum, A. C., Fraser, D. S., Slidders, W. and Henderson, R., (1962), *J. clin. Path.*, **15**,401.

This is a detailed article giving five methods for demonstrating fibrin. Fixation is discussed, the recommended fixative being a formalin-mercuric chloride combination. The principle of each technique is given and the article is well illustrated with colour photomicrographs. D. T.

Laminated Macrosections of Organs. A new Method for the Demonstration of Gross Pathology. Cote, R. A., Maynard, R. M. and Karthy, A. (1963), *Amer. J. clin. Path.* **39**, 54-58.

Macrosections of fresh or fixed organs are cut on an electric meat slicer. They are blotted and mounted by thermal lamination between thin sheets of plastic or plastic and a white backing card, using a copying machine. These macrosections can be used to demonstrate gross pathological abnormalities. They are easily transported and eliminate formalin vapour. D.T.

MICROBIOLOGY

Pasteurella multocida — **Human Infections.** Brodie, J. and Henderson, A. (1963), *Scot. med. J.*, **8**, 314.

The authors considered that an organism so prevalent in animals might be readily transmitted to humans. A careful watch on all incoming

specimens was undertaken and 255 sera examined for antibodies to this organism. 550 sputa were examined with negative results, but two exudates grew *P. multocida*. Antibody studies were not conclusive. Septicaemia is probably necessary to produce a diagnostic titre. T.E.M.

Preparation and Properties of Staphylocoagulase Toxoid. Harrison, K. J. (1963), *J. Path. Bact.*, **85**, 341.

This paper describes two methods of preparing a toxoid from free coagulase, a method of toxoid assay, some properties of the toxoid, and its use in the immunisation of animals. H.C.W.S.

Studies of Urinary Tract Infections in Infancy and Childhood. Winberg, J., Anderson, H. J., Hanson, L. A. and Lincoln, K. (1963), *Brit. med. J.*, **ii**, 524.

A report is given of the *E. coli* antibody response in infants and children with acute and chronic urinary tract infections. The methods included haemagglutination techniques, and these were related to antibody response in different types of urinary tract infection caused by coliform bacteria. H.C.W.S.

Antibiotics Revisited: Problems and Prospects After Two Decades. Jawetz, E. (1963), *Brit. med. J.*, **ii**, 951.

Doctor and technologist would profit from reading this lecture, originally delivered at the University of London. H.C.W.S.

MYCOLOGY

The Identification of *C. albicans* within Two Hours by the Use of an Egg White Preparation. Buckley, Helen R. and Uden, N. (1963), *Sabouraudia*, **2**, 205.

A method for identification of *C. albicans*, based on the Reynolds and Braude phenomenon in egg white, is proposed. By such means, "germ" cells may be observed at 37°C. after as little time as two hours. H.C.W.S.

Factors Influencing the Growth of *C. albicans*. Corman, R. H. and Goslings, W.R.O. (1963), *Sabouraudia*, **3**, 52.

Clinical observations and *in-vitro* experiments indicate the interaction of bacteria and *C. albicans*, with special emphasis on the latter micro-organisms. The glucose concentration is thought to be an important factor. H.C.W.S.

SEROLOGY

Immunological Techniques in the Diagnosis of Pregnancy. McCarthy, C., Pennington, G. W. and Geoghegan, F. (1963), *J. Obstet. Gynaec. Brit. Cwlth.*, **70**, 557.

A haemagglutination inhibition test has been compared with the Hogben test on urines from 485 cases of suspected pregnancy. Fewer false negative results were obtained with the H.A.I. test, which was also shown to give no false negatives in 280 cases of known pregnancy.

A Comparison of the Hogben Pregnancy Test with an Immunological Method. Barr, W. A. (1963), *J. Obstet. Gynaec. Brit. Cwlth.*, **70**, 557.

A haemagglutination inhibition technique (*Prepuerin*) was compared with the Hogben test on a total of 1,690 urine samples. The immunological method gave 97.4% agreement with the clinical diagnosis, while the biological test gave 99.2%. Most disagreements were where the *Prepuerin* test was falsely positive.

Laboratory Tests in Hydatid Disease: A Comparison of the Indirect Haemagglutination, Complement Fixation and Intradermal Tests. Arabatzis, G. and Papapanagiotou, J. (1963), *Bull. Wld Hlth Org.*, **28**, 266.

This study suggests that the indirect haemagglutination test should become a routine diagnostic method, in addition to some other technique—certainly in support of complement fixation tests. H.C.W.S.

The Health Department Examinations

FINAL — CERTIFICATE OF PROFICIENCY

(2, 3, 16 and 17 October, 1963.)

Written Paper (Bacteriology)
Answer all questions. *Time allowed 3 hours.*

1. Write a short essay on one of the following subjects:—(a) Nutrient Broth *or* (b) Bacterial Endospores and Sterilization Practice *or* (c) Agglutination Reactions *or* (d) Bacterial Variation. *(26 marks)*

2. Give a detailed account of the methods of examining a specimen of sputum for *Mycobacterium tuberculosis,* including the isolation and identification of the organism. Discuss the potential hazards in the various technical procedures, the equipment used, and the precautions to be taken. *(30 marks)*

3. Describe briefly *one* method of:—(a) testing for 'free' coagulase; (b) testing for oxidase; (c) testing for lecithinase; (d) preventing *Proteus vulgaris* from swarming; (e) distinguishing pneumococcus from *Streptococcus viridans;* (f) sending by post a sample of faeces for virus isolation; (g) anaerobic culture of sugar media; (h) staining spores; (i) making a streptococcal extract for Lancefield grouping; (j) disinfecting a contaminated bench. *(20 marks)*

4. Give a concise outline of the methods and media you would use to isolate:—(a) dermatophytic fungi from hairs; (b) leptospira from urine; (c) *Brucella abortus* from milk; (d) *Clostridium tetani* from dust. *(24 marks)*

Written Paper (Biochemistry)
Answer all questions. *Time allowed 3 hours.*

1. (a) Describe fully the *principles* involved in the estimation of total and direct bilirubin by the method of Powell. Indicate what is the purpose of each reagent used in the tests; (b) What types of bilirubin do these tests estimate and explain briefly how they may arise; (c) Discuss the approximate accuracy and the sources of error in the standard estimation using 0.4 or 0.5 ml. of serum; and in the micro estimation carried out on capillary blood using 0.05 or 0.1 ml. of serum; (d) Discuss the standards which can be used for calibrating a photo-electric colorimeter for bilirubin estimation. *(20 marks)*

2. Describe fully what precautions you would take (and why) in using a photo-electric colorimeter or simple spectrophotometer to ensure that the measurements obtained were accurate. *(20 marks)*

3. What laboratory tests for evaluating renal function do you know? Describe each briefly. Mention any precautions to be taken in carrying out these tests so that they may be reliable. *(20 marks)*

4. Write notes on:—(a) Anticoagulants used in biochemistry. Describe their action in preventing coagulation and their advantages and disadvantages. (b) Describe briefly the bromsulphthalein test for liver function, mentioning any precautions necessary to ensure accuracy. (c) A doctor has sent you a reddish or brownish-red urine specimen. Discuss briefly the possible causes of the colour. Indicate what tests you would carry out and what result you would expect in each case. (d) Define pH and write briefly on the pH scale. What is the difference between pH and titratable acidity? (e) How would you check a biochemical method to determine whether Beer's Law was obeyed? (f) How would you define an isotonic solution (as used in hospitals)? Do you know the standard method for accurately determining the isotonicity of a solution? *(40 marks)*

Written Paper (Blood Transfusion and Haematology)

Time allowed three hours.

A. Blood Transfusion.

1. Describe the tests you would perform on the plain (clotted) blood sample from a donor giving blood for, say, the fifth time in your transfusion service? Include all tests from the time of collection until his donation is considered ready for transfusion into a particular patient.

2. Answer each of the following questions using not more than five lines for each:—(a) What are the anticoagulant mixtures commonly used in blood transfusion? (b) Outline the essential difference between 'complete' and 'incomplete' antibodies. (c) What specimens are needed to investigate a suspected haemolytic reaction? (d) What tests would you perform on the cord blood of a suspected case of haemolytic disease of the newborn? (e) State the precautions necessary in transfusing group O blood to group A, B or AB patients.

3. (a) Discuss briefly the cell types which should be included in a panel of cells for screening serum specimens from ante-natal patients for antibody. (b) Discuss briefly the usefulness of the following techniques in the detection and identification of the more common 'immune' blood group antibodies:—(i) Enzyme-treated red cells; (ii) the use of albumin in solution; (iii) indirect Coombs' test.

B. Haematology.

1. Write brief notes on:—(a) Leucocyte peroxidase activity; (b) cyanmethaemoglobin estimation; (c) essential conditions for estimating erythrocyte sedimentation rate.

2. Set out, diagrammatically, the current theory of blood coagulation and outline the techniques of three tests which would give abnormal results in a case of classical haemophilia.

3. Discuss the inclusions which may be encountered in red cells in blood films stained by Leishman's stain.

Practical Paper (Bacteriology).

Time allowed 3 hours, with 30 minutes next day to complete examination of cultures.

1. Specimen 1 is a 1/10 dilution of a serum from a rabbit which has received a course of injections with a salmonella organism. Determine the H and O agglutinin titres. You are provided with nutrient agar and broth cultures of the salmonella organism. *(25 marks)*

2. Examine the cultures labelled 4, 5, 6, 7 and 8 by inspection and microscopy of stained smears. State the probable groups to which the organisms belong and write down *briefly* the next steps, if any, you would use in their identification. *(30 marks)*

3. Specimen 9 is a sample of guinea pig serum. Determine its complement titre. *(20 marks)*

4. Culture 10 is a direct plating of faeces on a selective medium. Complete the examination as far as time allows and report your findings (this question is to be completed tomorrow). Make a list of the media you need and hand this to the supervisor, who will supply your requirements as far as possible. This list will form part of your answer and will be marked. *(25 marks)*

Practical Paper (Biochemistry).

Time allowed 3 hours with 5 minutes to read the whole paper.

1. (a) Carry out a CO_2 combining power estimation on plasma specimen 1. Assume this plasma has been equilibrated with alveolar air immediately before you carry out the test. Very briefly describe the principle of this examination and explain briefly why it is necessary to equilibrate the plasma with alveolar air immediately before the test. *(25 marks)*

(b) Write notes on spot tests No. 2, 3 and 4. *(10 marks)*

2. Estimate the total serum protein, the serum albumin and serum globulin on specimen 5. Work out the albumin and globulin ratio. The serum T.N.P.N. on this specimen is 55 mg. per 100 ml. Show all your calculations in detail. *(32 marks)*

3. (a) Specimen 6 is a cord blood serum from a severely affected Rh infant whose cord blood bilirubin is 6 mg. per 100 ml., and whose haemoglobin level is 5 g. per 100 ml. Can you show, by spectroscopic or other tests, whether any other abnormal pigment is present. Describe in detail what tests you carried out, and your findings. Ask for any reagents required. *(12 marks)*

(b) Specimen 7 is a specimen of urine from a soldier who has completed a strenuous route march. Unfortunately the urine specimen was eight hours old when it reached the laboratory. Microscopic examination of the urinary deposit showed nothing abnormal. What is present in the urine? Describe any tests you carried out and give your findings. Ask for any reagents required. *(9 marks)*

(c) Write notes on spot tests 8, 9 and 10. *(12 marks)*

Practical Paper (Blood Transfusion and Haematology).

Time allowed three hours.

A. Blood Transfusion.

1. ABO and Rh (D) type the donor specimens 1 to 6, using a rapid technique.

2. Crossmatch donor specimens 1 to 4 inclusive with the serum of the patient X.

B. Haematology.

1. On the blood specimen with E.D.T.A. anticoagulant marked Z, estimate the following:—Haemoglobin; packed cell volume; M.C.H.C.; leucocyte count; reticulocyte count; differential leucocyte count; comment on erythrocyte, leucocyte and platelet morphology.

2. Carry out an osmotic fragility screen test on the heparinised blood sample P.

3. Comment on the six blood films numbered 1 to 6.

Successful Candidates

Cox, Miss M. H. F. ... Christchurch
Cross, L. G. ..... Gisborne
Elliott, B. J. P. ..... Auckland
Gratten, M. J. ..... Christchurch
Harding, Miss J. L. ..... Auckland
McLaughlin, P. O. ..... Rotorua
Nicholas, R. J. ..... Palmerston Nth.
Phillips, Mrs D. M. ..... Ashburton
Silvester, Mrs D. E. ..... Opotiki
Tripp, Miss E. H. ..... Wellington
Williams, A. H. ... Palmerston Nth.

Twelve candidates sat the examination, three in one subject only. Eleven candidates were successful.

Book Reviews

A Synopsis of Blood Grouping Theory and Serological Techniques. A.D. Farr, F.I.M.L.T., A.I.S.T. William Heinemann Medical Books Ltd., London, 1963. 108 pages. Price in U.K., 21s 0d.

Just let a specialist in any field take up his pen to compile a concise yet knowledgeable and helpful guide to his subject for beginners, and the problem he faces from the start is that of knowing how much to leave out. The author of the book under review succeeds tolerably well in his purpose, but if he appears to have chosen unwisely in some instances, it may be because the science of blood group serology has become so complex in recent years that no two of its experienced practitioners can agree in every particular about which of its aspects should be regarded as essential knowledge.

Brevity is expected in a book purporting to be a synopsis, but we are entitled to expect the most up-to-date information in a recently published book and this is where Mr Farr makes his worst mistake. To have embarked on a treatment of 'private' blood group antigens without the intention of recognising the work of Cleghorn is difficult to excuse. The table of low-incidence antigens is outdated; it is not true that no figures have been published regarding the incidence of the Wright antigen in Europeans; and to reiterate the fallacious belief that anti-Wr^a is associated, in particular, with anti-E, is to revive a long-discarded notion and to sacrifice an opportunity to call attention to its occurrence as a contaminant in a significant proportion of all immune typing sera.

This book combines simply explained fundamental principles with easy-to-consult technical details, but bearing in mind the class of reader at which it is directed, its weakness is its occasional tendency to mislead as exemplified in the sentence: 'Several examples of anti-Fy^a have been discovered, and all have been responsible for haemolytic transfusion reactions' from page 29. Occasional typographical errors are to be expected in almost any printed work, so perhaps we may make due allowance for the fact that the Sw^a antigen is given as Swain instead of Swann; the context will lead us to view with suspicion the solemn assertion that some examples of anti-c and anti-e also contain anti-F, but the reader who heeds the counsel (on page 46) to prepare his trypsin suspension in N/20 NaOH is courting disappointment and failure.

To further prolong the catalogue of this book's faults would be to place undue emphasis on them, and this would be unfair. The overall impression created is favourable in spite of the shortcomings mentioned. The value of such a work to the trainee technologist coming to the blood grouping laboratory for the first time in undeniable, as is the fact that it presents the theory of the subject in terms that are simple to understand. This is not a book one could recommend to the experienced senior technologist in a specialised blood grouping laboratory, but it will prove a very valuable guide to those preparing for the intermediate and final examinations and has its place on the bookshelf of any hospital laboratory.

J.C.

Biological Staining Methods. George T. Gurr, F.R.I.C. 7th Edition. G. T. Gurr Ltd, London, 1963. 116 pages. Obtainable from G. T. Gurr Ltd, 136 New Kings Road, London S.W.6, at 7s post free.

The bulk of the techniques in this book are for use in Histopathology but there are approximately fifty staining methods for use in Bacteriology and about twenty for use in Haematology. There is a compact history of the Romanowsky stain and brief details of histological embedding methods and materials. It also contains the usual formulary and tables giving solubilities of dyes and a few chemicals etc.

More references are included with the techniques than in the previous edition but there are still quite a number of methods given with neither reference nor results. A silver technique is included to demonstrate reticulin but there are none given to demonstrate components of the central nervous system.

Due to the large number of techniques and lack of explanatory details the book cannot be recommended for students, but the more experienced technologist will find it useful to refresh his memory and for comparison of techniques.

D.T.

Council Notes

A Council meeting was held at Wellington Hospital on Saturday, December 14, 1963. Present were Mr H. G. Bloore (in the Chair), Misses H. M. Bond and J. Mattingley and Messrs J. Case, H. E. Hutchings and J. D. R. Morgan. Apologies were received from Messrs. G. R. George and R. T. Kennedy and Messrs M. McL. Donnell and D. J. Philip were prevented from attending by transport difficulties.

New Rules

Drafts of the newly amended rules of the Institute were considered by the Council and the Secretary was instructed to make arrangements for these to be reproduced by the xerox process. It was agreed that all members of the Council should be afforded the opportunity to check the proofs, but it was hoped that the new Rules would be ready for distribution to members with the April Journal.

Associateship Diplomas

The lay-out of the proposed diplomas to be awarded to Associates and Fellows of the Institute was considered and the wording approved. The Secretary will arrange for the printing of four hundred copies of the diplomas in the immediate future and, in due course, members qualified for Associate membership will be invited to apply for their diplomas. Fellowships will be introduced at a later date, when the advanced examination is available.

Life Members

On the instructions of the Annual General Meeting, the Council considered the question of life memberships of the Institute. It was decided that this question should be reviewed at intervals, but the principle was agreed that life membership should not be awarded indiscriminately and that this honour should be reserved for conferment on members who had rendered outstanding service to the profession.

Examinations

The President reported that the new examination syllabuses were now almost ready for final approval by the Director-General of Health, but that there seemed to be some delay over the part relating to the advanced examination in Haematology and Blood Transfusion Technique. It was hoped that the syllabuses would be complete and ready for circulation before March, 1964, in order to allow twelve months notice of their introduction as the basis for the 1965 examinations. Now that a comprehensive schedule of training is to be introduced, it seems reasonable that a higher standard of marking in the final examination will be justified; and it remains to be seen whether or not the syllabuses will prove practicable as a guide to examiners. The possibility of specialisation after the intermediate examination may have to be given consideration later.

Salaries Advisory Committee

The President advised that submissions on salaries will require to be given more detailed consideration in the future, and that adequate justifications will have to be made out before placing any submissions before the Committee. The recommendations of the November meeting of the S.A.C. have gone forward for ministerial consideration and an official pronouncement can be expected in the next few months.

Appeal and Arbitration Machinery

The sub-committee appointed after the Annual General Meeting had devoted some attention to the matter contained in its terms of reference, but had met with very little hope of success. The position of the Registered Nurses Association is not strictly comparable with our own, and it was worthy of note that appellants before the Public Service Appeals Commission (for example) were seldom successful in their appeal. Such pleas were allowed in only $2\frac{1}{2}\%$ of cases and, whatever the disadvantages of the present system, whereby appeals were heard by the

same body whose decision was being appealed against, it had to be admitted that the Grading Committee was in the best position to appreciate all the facts of the individual's case.

Examination Board

In expectation of a Health Department request for nominations for the Institute's representatives on the Medical Technologists Board, it was agreed that the three nominees should be Messrs H. G. Bloore, L. Reynolds and G. McKinley, with Miss J. Mattingley and Mr H. E. Hutchings in reserve. If it is the intention of the Department of Health to call for fresh nominees annually, it was considered that it would be in the Institute's best interests if its representatives should each be re-nominated annually for three years and then replaced. With one representative retiring annually, this would permit continuity and at the same time make way for new blood at frequent intervals. The principle that all branches of the work should, wherever possible, be represented, was agreed.

Meetings of Groups of Members

In referring to a proposed meeting of South Island members at Timaru in April, the President gave great weight to the value of such gatherings, but expressed some concern at the growing tendency to dignify them with the title 'conference'. While endorsing the principle that meetings of this kind were to be encouraged, the Council agreed that the name 'conference' should be reserved for the annual national meeting of members, and suggested that the term 'seminar' may be more appropriate for such assemblies as the one mentioned.

New Members

The following were admitted to membership of the Institute:

Collins, B. S. ..... Ruakura
Collins, Mrs F. ..... Dunedin
Drewitt, Miss V. A. ..... Rotorua
Hooker, R. O. ..... Hastings
Jarman, N. E. ..... Auckland
Martin, Miss F. S. ..... Dannevirke
Morrison, Miss J. ..... Dannevirke
Reid, I. G. ..... Kawakawa
Toplis, Miss B. ..... Kaikohe
White, B. M. ..... Auckland

It was considered just that members accepted at Council meetings held after October 1 each year should, on payment of their subscriptions, be considered financial for the following financial year, Accordingly, the above members will be financial for 1964/65 on payment of their initial subscription.

The Journal

The expenditure of approximately £188 of the Institute's funds on the printing and publication of the Journal for 1963 was considered justified, and the editor's request for an advance of a further £150 at the beginning of the next financial year was approved. The campaign to obtain more advertisers was meeting with moderate success and it was hoped that the revenue from this source may be increased during 1964. With enough material already in hand for the April and July issues, the editor reported that the increased support had been maintained and that there seemed every reason to expect that the Journal could be maintained at its present size.

Copies of volumes 11 to 16 inclusive will be bound for the official records of the Institute and commencing with volumes 17 and 18, binding will be carried out every two years.

A new feature commencing in 1964, will be the regular publication of a list of journals received on an exchange basis, together with lists of the contents of those issues received recently.

Branch Reports

AUCKLAND

(Secretary: I. C. King, 111 Cliff View Drive, Green Bay, Auckland.)

1963 proved to be another successful year of activity, during which

numerous excellent speakers made themselves available to address our monthly meetings. Climax to this year was another one-day seminar, which drew an attendance of some 80-90 technologists from Tauranga to Rotorua and Whangarei. A very good attendance of 27 visitors, representing six laboratories outside the sphere of the local branch, attests to the popularity this annual event is attaining. It is particularly heartening to note that of the 16 papers presented at this meeting, all but two were presented by technologists — the remaining two being by pathologists.

The following are some of the speakers and their subjects from the regular monthly branch meetings:—

Dr A. W. Liley *Haemolytic Disease of the Newborn.*

Mr Nelson (later Dr Nelson) *Services Provided by the Government Laboratory.*

Mr Brown (of the St. John Ambulance Association) *Modern Methods of Resuscitation.*

Mr J. Pybus *Cave Exploration.*

There was also a paper on *Brewery Chemistry* provided by a chemist from N.Z. Breweries and a talk by Dr F. Sims on his trip overseas. Several films of technical and non-technical interest completed the activities for the year.

At the Annual General Meeting in October, the following executive was installed for the ensuing twelve months:

Chairman: Mr J. Walsh.
Secretary: Mr I. King.
Treasurer: Mr W. Wiggle.
Committee: Mr T. E. Miller, Mr R. T. Kennedy, Mr J. Holland.

I.C.K.

DUNEDIN

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

Dr A. Veale of the Genetics Research Unit addressed the September Branch Meeting on the subject: *Polyposis and Genetics,* great interest being indicated by the enthusiastic question session that followed.

Officers for the 1963/64 period elected at the Annual General Meeting on October 2 were:

Chairman: Mr B. Main.
Secretary: Mr E. K. Fletcher.
Treasurer: Mr J. Rees.
Committee: Mr R. D. Allan, Mr J. D. R. Morgan, Miss J. M. Edgar.

The November Branch Meeting was held at Invercargill, where two papers from the Conference were re-presented, and Mr and Mrs G. C Thompson received members at a social evening following the meeting.

Branch activities for the year closed with the Christmas function held at Glenfalloch.

E.K.F.

The Rex Aitken Memorial Prize

Members are reminded that this award of £25 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 1963.

Intending entrants should submit three copies or reprints of their work to the editor of this journal, to reach him not later than May 15, 1964.

The Library

(Librarian: J. Rees, Pathology Department, Medical School, Dunedin)

Periodicals received since September 1963.

Amer. J. med. Technol. Volume 29, No. 5 September/October 1963.

Contents: A Simple Method for Demonstration and Enumeration of Human Chromosomes; Simplified Technic for Preparing a Stable Bilirubin Standard; The Development of Methods for Determining Antibiotic Susceptibility; Crossmatching in the Presence of Autoantibody; Regulating pH of Gas for Tissue Culture Propagation; Demonstration of Leukoagglutinins — A Simplified Screening Method; A Microcapillary Tube Technique for Estimation of Serum Gamma-Globulin; Evaluation of an Ultramicro Method for Blood Glucose Determination; Reproducibility of Serum Protein Fractions by Cellulose Acetate Electrophoresis.

Volume 29, No. 6 November/December 1963.

Contents: Quality Control in Medical Technology; Colorimetric Tests Read by Colour-Blind People; Problems of Hospital Asepsis As They Concern the Laboratory; The Genus *Aeromonas* — Methods for Identification; Observations on the Sickling Phenomenon; Detection of Abnormal Haemoglobins; Apparent Depression of Serum Globulin Caused by a Change in Method; A Plastic Sputum Bottle for Collection and Concentration of Specimens; The Direct Reading Newborn Bilirubin Method as Used in our Routine Clinical Laboratory.

Ann. Med. exp. Biol. Fenn. Volume 41, No. 3. 1963.

Abridged Contents: Microdetermination of Acetone Bodies in Blood and Urine; The Alizarin Test and Its Mechanisms; Forensic Chemical Identification of Meprobamate by Thin Layer Chromatography in the Presence of Sedatives Other Than Barbiturates; Adamis Method for the Determination of Plasma Fibrinogen; Extraction of Serum Inositides and Other Phosphatides; Some Implications of the Use of Activated Papain for the Screening of Blood Group Antibodies; Notes on the Immunological Pregnancy Test, the Toad Test and the Rat Test for Pregnancy; The Bacteriostatic Activity of Wood Tar; The Action of Gaseous Ethylene Oxide on the Cultures of Mycobacteria; The Occurrence of Atypical Acid-fast Bacilli in Human Faeces; Determination of the Sensitivity of Mycobacteria to Isoxyl.

Volume 41, Suppl. 1, 1963.

Contents: Virus and Hereditary Resistance *in Vitro*.

Arch. Inst. Pasteur Hellen. Volume 9, No. 1. June, 1963.

Contents: Modern Conception and Genesis of the Leptospirae; On the Diagnosis of Myelofibrosis; Review of Haematological Work Conducted in the Newborn Unit of the Alexandra Hospital and the Paediatric Unit of the Children's Hospital "Aghai Sofia"; Haemophilia in Greece. (All articles in French with English summaries).

Aust. J. biol. Sci. Volume 16, No. 4. November 1963.

Canad. J. med. Technol. Volume 25, No. 5. October 1963.

Contents: Genetic Markers and the Identification of Virus Strains; The Detection of Iso-leukoagglutinins and Their Clinical Significance; A Modification of the Pool and Robinson Quantitative Antihemophilic Globulin Assay; A Study of Serum Proteins by Paper Electrophoresis in Patients with Malignant Tumors.

Volume 25, No. 6. December 1963.

Contents: Problems in the Laboratory Diagnosis of Tuberculosis; Decompte des Plaquettes a l'aide d'un Compteur Electronique; the Coagulation Time; A Comparison of a Macro and a Micro Method for Blood pH Determination; A Simple Method of Preparing Calibrated Dropping Pipettes for Variable Bacterial Counts; An Attempt to Use

Polyethylene Glycols as Routine Histological Embedding Media.

Filter. Volume 35, No. 2. June 1963.

Contents: Principles and Applications of Paper Partition Chromatography; An Investigation of Beta-Hemolytic Streptococci by Biochemical Methods; Administering the Laws and Regulations Which Pertain to Clinical Laboratories and Their Personnel.

Volume 35, No. 3. September 1963.

Contents: Some Mycotic Infections Found in California; Lysozome Therapy in Malignancy.

J. med. Lab. Technol. Volume 20, No. 4. October 1963.

Contents: The Preparation of Slides of the Exfoliated Cellular Material in Body Fluids; A Non-drying Fixative for Use in a Cervical Smear Diagnostic Portal Service; Detection of Volatile Fatty Acids Produced by Obligate Gram-negative Anaerobes; Some Factors Affecting a Disinfectant Suspension Test; A Silver Impregnation Technique for Peripheral Nerves in Frozen Sections of Decalcified and of Soft Tissues of the Oral Cavity; A Note on the Estimation of Copper; The Effect of the Anticoagulant on Protein Estimation; A Rapid Method for Decalcification of Bone for Histological Examination Using a 'Histette.'

Lab World. Volume 14, Nos. 8, 9, 10, 11 and 12. August-December 1963. Volume 15, No. 1. January 1964.

Med. Technol. Austral. Volume 5, No. 3. July 1963.

Contents: Serum Protein Paper Electrophoresis in the Dog: Towards the Genetic Code; Paper Electrophoresis with Zeiss Jena—Instrument ERI 10.

Microbiologia (Buc.). Volume 8, No. 3. May-June, 1963.

Abridged Contents: Schick's Test; *Contributions to the Study of the Epidemiology of Intestinal Parasitosis in the City of Bucharest; A Case of Three-fold Infestation of the Body with *Metastrongylus elongatus, Taenia saginata* and *Enterobius vermicularis;* *On In-Hospital Infections Due to Pathogenic *E. coli;* A Fatal Case of Septicaemia due to *Cl. perfringens;* Investigations on the Follow-up Period of the Lowenstein-Jensen Medium Seeded with Sputum for the Isolation of Koch's Bacillus; Preserving and Staining of Coprologic Smears by the Polyvinyl Alcohol-Trichrome Method; *The Cultivation of Non-Haemolytic Pneumococci and Streptococci in TSIEM Glucose Broth; Contributions to the Study of Sodium Hippurate Hydrolysis by Streptococci. (All articles in Rumanian, but those marked * have English summaries).

Volume 8, No. 4 July-August, 1963.

Abridged Contents: The Guinea-pig in Scientific Investigation and Biological Tests; The Chemical Bases of Stains in Histochemistry; Enteropathogenic *Escherichia coli* Serotypes Isolated in the Banat; Contributions to the Study of the Incidence Rate of Enterococci in Meat Products; *Pathogenic Mechanisms and Clinical Manifestations Found in Lambliasis; *Investigations on the Influenza Virus Strains Isolated During the 1962 Epidemic in Jassy; *The Biologic Bases and Clinical value of McLagen's Test in Viral Hepatitis; *Epidemiologic Study Concerning the Efficiency of the Pertussis Component in the Trivaccine Prepared in the "Dr I. Cantacuzino" Institute.

Volume 8, No. 5. September-October, 1963.

Abridged Contents: Messager Ribonucleic Acid; Bacteriocides; *Brucellosis as a Factor of Occupational Morbidity among Doctors and Veterinary Technicians; *Staphylococcal Scarlet Fever; *Considerations on the Presence of Streptococci in Animal Foodstuffs; *Epidemiologic and Clinical Remarks on Anthrax in Cluj During the Last 13 Years; Preparation of the Cardiolipin Antigen and its Testing by the Complement Fixation reaction.

Volume 8, No. 6. November-December, 1963.

Contents: This issue is devoted to aspects of the work of the late

Professor Ion Cantacuzino and of the Institute established in his memory. (All in Rumanian with no English translation).

New Istanbul Contr. clin. Sci. Volume 6, No. 1. January 1963.
Abridged Contents; Congenital Stuart Factor Deficiency.
Volume 6, No. 2. April 1963.
Abridged Contents: A Review of Hepatic Porphyria; Erythrocyte Phospholipid in Periodic Disease; Specific Uric Acid Estimation by the Carbonate Method Without the Use of Uricase; Thalassaemia—Haemoglobin H Disease — Study of a Turkish Family.
Volume 6, No. 3. July 1963.
Contents: The Transfusion of Platelets; Intracellular Glycogen in Lymphoproliferative Diseases; Platelet Osmotic Resistance Test Using the Electron Microscope; A Case of Haemophilia A with Circulating Anticoagulant; Alkaptonuria; A Case of Factor V Deficiency.

N.Z. Hospital. Volume 16, No. 3. January 1964.
Rev. Viernes med. Volume 14, No. 2. May-August 1963.
S. Afr. J. med. Lab. Technol. Volume 9, No. 2. June 1963.
Contents: A New and Safer Cro-smatching Procedure; Assay of Enzymes in Human Materials.
Volume 9. No. 3. September 1963.
Contents: Neutrophil Alkaline Phosphatase Activity in Health and Disease; Ethylene Oxide Sterilisation under Normal Pressure and Temperature.

The Wellcome Trust

£800,000 FOR MEDICAL RESEARCH

During the year ended August 31, 1963, a total of more than £800,000 was allocated by the Wellcome Trustees for medical and veterinary research projects.

In the six months immediately prior to August 31, major awards included £53,000 to the University of Leeds School of Medicine for research on cardiac physiology; £40,000 to the Tata Institute for Fundamental Research in Bombay for molecular biology research; £26,000 for the construction of laboratories for biological research at the Beilinson Hospital, Petah Tikvah, Israel; and £26,000 to the Harvard School of Public Health at Boston for laboratories for experimental pathology, with especial reference to research on schistosomiasis.

Post Graduate Course in Medical Laboratory Technology

Conducted under the auspices of the Auckland Hospital Board and the Department of Health, a course was held for senior technologists at Green Lane Hospital in October 1963.

The programme was organised by Mr I. Cole, Charge Technologist at Green Lane Hospital, and extended over three days, covering recent advances in many aspects of medical laboratory technology, technical training, discussions and a visit to the Central Sterile Supply Department at Auckland Hospital.

The course was attended by senior technologists from Kaitaia, Kawakawa, Whakatane, Dargaville, Tauranga, Rotorua, Auckland, Hamilton, Taumarunui and Whangarei.

Vacancy

Royal Perth Hospital, Western Australia

SENIOR MEDICAL LABORATORY TECHNOLOGIST IN SURGICAL RESEARCH

Applications are invited for Medical Laboratory Technologists to fill the above post.

Royal Perth Hospital is the main teaching hospital associated with the Medical School of the University of Western Australia and has a bed capacity of up to 850 beds.

Duties of the appointment include participation in surgical research, supervision of research theatre activities, technical staff, etc., and the appointee, in addition, will be required to assist in the instruction of trainee technologists.

Applicants, who should be holders of a Degree in Science and/or the Diploma of a recognised Institute of Medical Laboratory Technology, should have experience in a research unit associated with a major teaching hospital.

SALARY: Within the range of £A1,959—£A2,211 per annum, commencing rate according to qualifications and experience.

The Hospital will meet the cost of a first-class sea passage for appointee and family (children up to 16 years of age) and an amount of up to £A200 for the cost of removal of personal effects (excluding furniture and motor vehicle), or an amount of up to £A25 in the case of a single person, on the appointee entering into a bond to remain in the Hospital's service for a period of three years.

Applications should give details of qualifications, experience, present post held, research interests, furnish the names and addresses of two professional referees and enclose a recent photograph.

CLOSING DATE: 31st May, 1964.

JOSEPH GRIFFITH,
Administrator.

WANTED TO BUY

Good, Second-hand Laboratory Equipment, e.g., Microtome, Centrifuge, Incubator, etc., to be used in setting up new laboratory in a Mission Hospital, Lucknow, India. Please contact as soon as possible: S. ENTWISTLE, C/o Pathology Department, Medical School, Dunedin.

Contents

PRINTED BY THE EVENING STAR CO. LTD.