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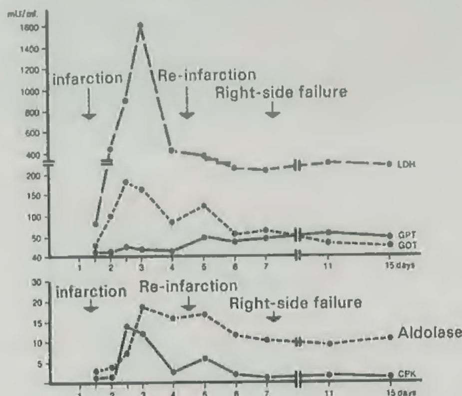

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Volume 28, No. 3 November, 1974

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T. H. PULLAR MEMORIAL ADDRESS

Medical Technology Education: Where is it Going?

H. E. Hutchings

Health Sciences Department, Central Institute of Technology

Presented at the 30th Annual Conference of the New Zealand Institute of Medical Laboratory Technology, New Plymouth, August 1974

Mr President, distinguished guests, ladies and gentlemen,

May I first acknowledge the honour bestowed upon me when I was asked to give the T. H. Pullar address. It is of tremendous personal significance to me to be able to recognise a a man who was a friend, a benefactor, a guide; a man who could, with wit and understanding, make one ' stand tall ' or look at oneself with belittling self-criticism.

Not surprisingly I have chosen to review the subject of ' Education '. The education and training of the medical technologist is a subject which was of particular interest to Thos. Pullar and it is now, of course, one of considerable concern to me.

I have re-read with interest and some surprise much that has been published over the years. Surprise came at the amount that had been written specifically on Medical Technology Education in New Zealand. The interest was generated by what was said, about a decade ago. Some of the proposals, and the concepts, put forward then have been established—in part. Others are still being propounded today, with remarkably little change.

What has occurred, in review, has been evolution and I suppose one must agree, at least in part, with the statements of one member of your Council who states that ' evolution is always wiser than revolution '.

If, however, what is being proposed today is in essence that mooted ten years ago then the evolution has been dilatory.

A recent upsurge in the continuing interest in education by the Institute Council has brought a reconsideration of the various programmes recommended. The subsequent reports have much in common, but still a somewhat quiescent state remains. If we have not already done so we must force ourselves to wonder at the cause of the impasse.

The N.Z. Certificate in Science, now in its fifth year, has itself undoubtedly reduced the drive towards development of any further programmes. It has been reasonable, I suppose, to assess the practicality and success of this as a programme for a basic qualification before proceeding further.

It has disturbed the tranquillity of the establishment and traditionalists have sought and, without too much difficulty have unearthed some valid criticisms. While the purpose of the criticism has been to cry for the good old days, it has instead, when put beside the only too evident advantages, generated a desire to modify, to extend and to improve.

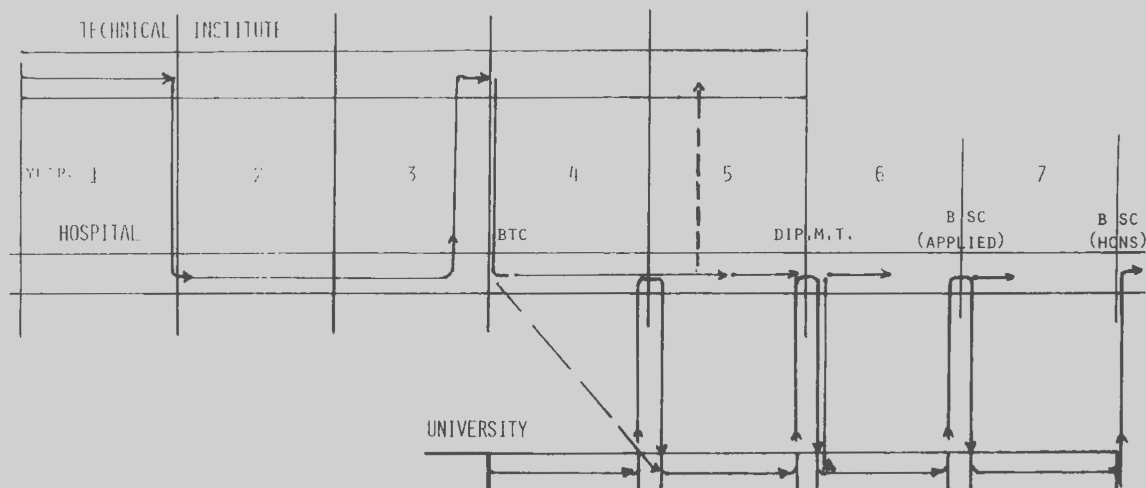
The most readily recognisable problem with the present education programme in the day release classes would be the difficulty the student has to concentrate after a full day's work, particularly when the subject required to be studied seems to have a complete lack of association with what has been performed in the laboratory during the day.

This lack of relativity is accentuated when the study is dropped into odd hours during a normal working day. These intermittent working hours further lead to tension within the laboratory as a result of total disruption of the continuity programme of the department. Combine these problems with a need to supply a 24-hour service and you create a medium in which ulcers, nervous breakdowns and coronaries are grown. Students fare better under the block course but the pressures of the intensive study of such a course is a severe penalty to pay for the privilege, particularly when in the early stages there still seems to be an apparent lack of relevance between the basic sciences and the vocation adopted by the student.

Considerable thought has been given by a number of people to all sorts of alternate proposals and concepts, most of them with large portions in common.

I would, at this point, like to briefly outline three of these proposals diagrammatically.

The first is largely a modification of the present system. This proposal (Fig. 1) envisages an initial three-year period where the student enters the Technical Institute with University Entrance or higher in selected subjects for a



PROPOSAL 1: Possible pathway (⟶) followed by a student from a Technical Institute to a Hospital and/or University over a seven year period.

full academic year to cover the basic sciences and learn the rudimentary skills in order to enter the laboratory at the commencement of the second year and begin some practical experience. Toward the end, or at the end of the following two-year period, he returns to the Institute for a minimum of one term to superimpose on that practical experience theoretical knowledge not before applicable. This would bring him to a level at least equating with the present NZCS and would be the basic registrable qualification.

The following two years would be further clinical experience in an appropriate laboratory with the opportunity to apply and attend sandwich courses in a specialist discipline. Applicants would be selected by merit and, on successfully completing such a course and the appropriate examinations, would be awarded a Diploma in Medical Technology.

Two alternative channels are possible in this proposal. Selected candidates could be offered scholarships at the basic qualification level to enter an appropriately designed degree course at the commencement of Year II or, under similar criteria, on the completion of the Diploma enter the same degree course at the commencement of Year III.

The second course to be outlined is one which is not too dissimilar from that first described.

It would be fair and, indeed, pertinent to quote the introductory paragraph given to this proposal: it is as follows.

> 'The basis of this full-time course should be the provision of sufficient basic sciences leading to Medical Laboratory disciplines with as much related practical experience as can be given. The level of the course should be pitched at providing a general medical laboratory technology practitioner who could, under supervision, turn his hand to routine techniques in any laboratory. The academic level would need to reach probably the present Part II level."

This course (Fig. 2) would be of four years duration and would comprise two years' full-time attendance at a teaching institute followed by two years in a recognised training laboratory. During this two years in the training laboratory it is recommended that 1,000 hours be spent in each of the disciplines, haematology, clinical chemistry, microbiology and immunohaematology (during which a minimum of 160 hours of cross-matching for clinical purposes be performed as laid down by the Medical Technology Board). Students completing this course would be eligible, if successful, for a Basic Training Certificate in Medical Laboratory Technology, and Registered Laboratory Technicians.

The course, of course, envisages the eventual withdrawal from the New Zealand Certificate of Science (Paramedical) under the auspicies of the Technicians Certification Authority.

The proposal emphasises that not all labora-

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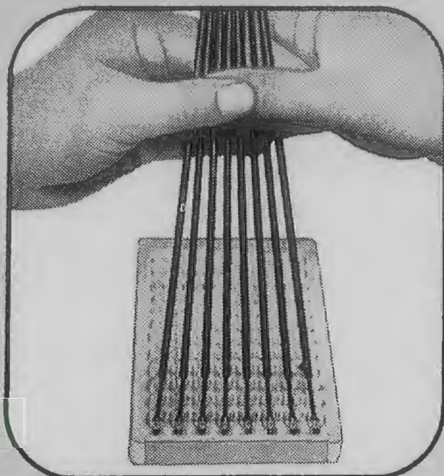

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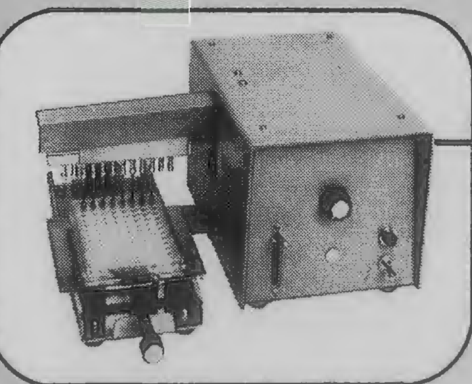

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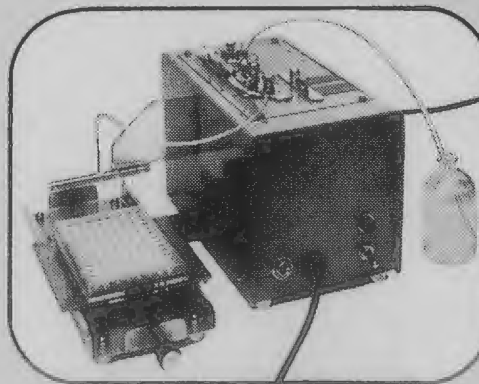

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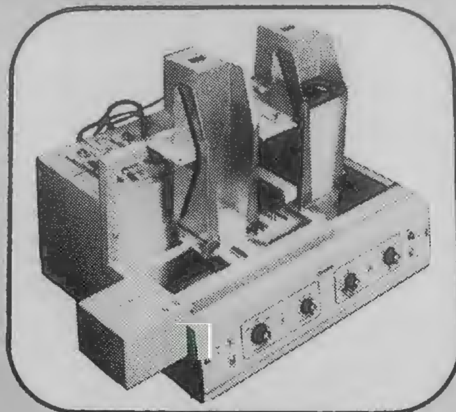

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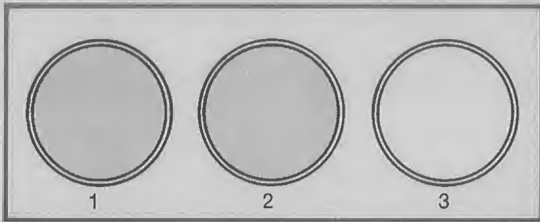

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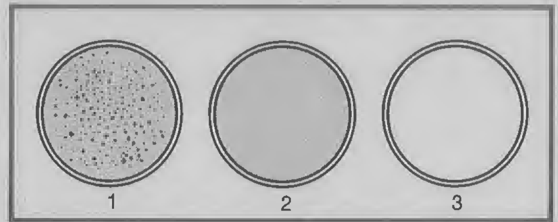
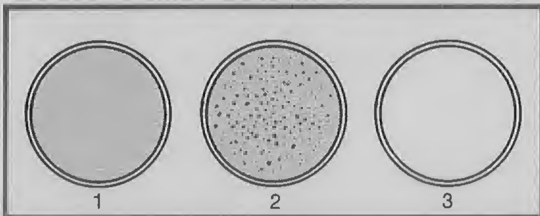
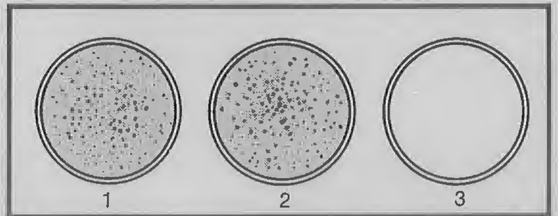


Stronger agglutination in circle 1 than circle 2:
positive for Infectious Mononucleosis.



Stronger agglutination in circle 2 than circle 1:
positive for Forssman antibodies,
negative for Infectious Mononucleosis.



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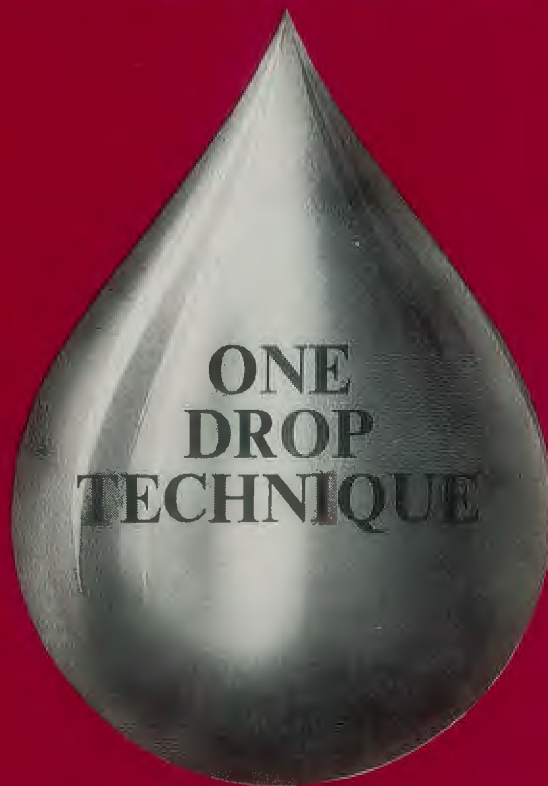

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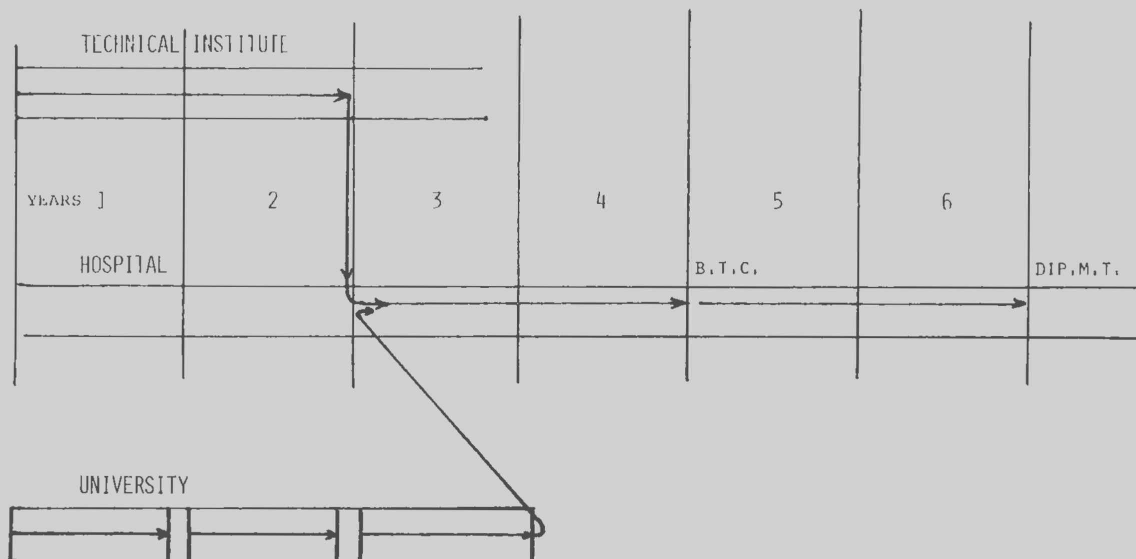


PROPOSAL 2: Possible pathway (⟶) for a student from a Technical Institute to a Hospital or for a B.Sc. graduate.

tories should be recognised as teaching laboratories. A list would need to be compiled and would be made up largely from the metropolitan and medium-sized hospitals.

A further, advanced course, designed to fulfil the recognisable need for specialists in a variety of areas is also conceived. The technicians would be required to apply for technology training positions in selected, recognised training laboratories with the successful applicant completing a minimum of 3,500 hours in the discipline of selection. The level of achievement would be that of the present Part III examination.

The examinations on completion of the course would be theory and oral only and would earn for the successful candidate a Diploma in Medical Laboratory Technology and would justify the diplomate earning a salary of a staff technologist.

Three criteria are recommended to govern this concept:

(a) Because the students are applying for technology training positions, they will be required to demonstrate their ability and be appropriately motivated before selection. This is obviously seen as a means of control of an excess in the number of laboratory technologists.

(b) The senior course would be an in-service one, similar in design to that of a pathology registrar.

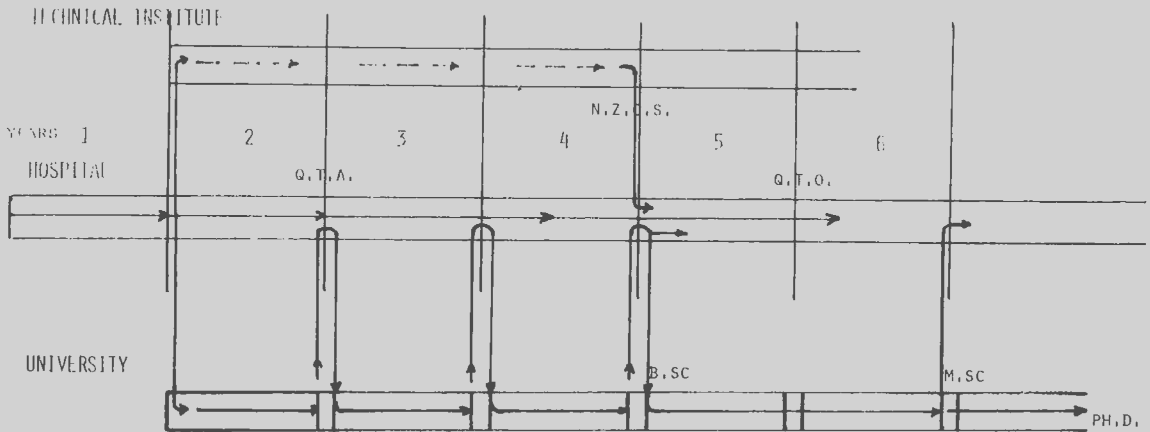
(c) The students would be required to teach themselves, but might be guided by tutorials and some lecturing where appropriate. Hopefully their practical training would be similarly guided.

As indicated even more rigid control of training laboratories is envisaged, such laboratories being selected on application and on having demonstrated certain minimum standards. Such recognition could well be withdrawn if the minimum standards were not maintained.

University graduates wishing to take a Diploma would be required to have obtained the Basic Training Certificate first and would have no exemption from the Medical Technology sections of the course but would have cross-credits for the basic science ingredients of the Basic Training Certificate.

The third proposal to be outlined, in some respects differs radically from those already described.

This concept (Fig. 3) is based on the already well-established staff establishment by technical assistants in some laboratories. It suggests that the medical laboratory technologist, as he is presently trained and qualified, is becoming



PROPOSAL 3: (⟶) indicates three alternate pathways for students; training in the Hospital, N.Z.C.S. program in the Technical Institutes or B.Sc. at University.

unsuited to modern medical laboratory requirements. It proposes that laboratory personnel should fall into two broad categories.

The first category is the university graduate, while the second is the laboratory assistant.

The proposal recommends that all laboratory workers should begin as laboratory assistants aiming initially at the qualified technical assistants' examination. The small number of intelligent and competent workers in this group should be channelled either into a university cadetship programme or into one of the already existing N.Z. Certificate in Science courses after one year in a laboratory.

A small number of highly selected people would be processed through a bachelor of science degree on a bonded cadetship basis. While attending university they would be required to work in a clinical laboratory during vacation periods and be given a continuing exposure to the clinical situation throughout the year. The units which the students would undertake would be those of a normal university course plus a selection which would be studied with the approval of the parent laboratory. Progress through the course would be continually assessed and the ability of the student to ultimately fill a senior post would be reviewed. Any failure to achieve satisfactory progress could be reason for terminating the contract, either during or at the end of the cadetship, the unsuccessful student being either returned to the laboratory assistant stream or advised to seek re-employment. It is felt that the selection of cadets after employment in a hospital laboratory for one year would minimise this need for re-direction.

With the full implementation of such a scheme, based on the university, the present training system could then be phased out. Staff technologists at present employed could be regarded as equivalent to the cadetship trained technologist for salary and grading purposes. Staff at present in training would be given access to the cadetship scheme and appropriately graded medical technologists should be offered, as with the successful cadetship students, opportunities to proceed to higher academic qualification.

All proposals accept the use of professional education in appropriate forms.

The most readily recognisable need in the education programme then would seem to be that at least part of the course, and preferably at its commencement, should consist of full-time teaching at some educational institute. Administrative organisation within the laboratories would certainly be eased. A more applicable and related syllabus could well be devised with a more amenable learning atmosphere. While the very essential basic sciences could be retained and be more appropriately integrated and directed towards diagnostic laboratory work. The students hopefully might then enter laboratories familiar with the language, with a sound background and with some immediately useful abilities.

It's a good beginning, perhaps more than a

beginning, perhaps the basis on which ideas could be built leading to an educational programme worthy of recognition as a registrable basic qualification. It could be the very necessary foundation for the tier of personnel required to maintain the diagnostic laboratory service.

From it could grow other, more advanced, more specialised personnel. Personnel to modify, direct, manage and to accept responsibility.

Why then has so little been achieved? Why has there been so much difficulty in melding an overall programme? Some say it is too soon to start altering things. At least one educational authority has made this statement. On the other hand, if there are difficulties, why perpetuate them?

Some say the programmes are too ambitious and over-education will result. This is rubbish! Is there such a thing as over-education? It requires individual ability and drive to pursue any educational course. There are the recognisable opportunities to step aside from further education in all the proposals. Supply and demand add further controls.

Is there fear of or pressure from outside? Not the pathologist surely? The better the technologist the better his laboratory. Besides, through established committees and the Medical Technology Board, communication is better now than it has ever been before.

The technologist is already part of an alternate tertiary education system whose worth is recognised by the universities, if not all their products.

No, it is something more fundamental, something more basically simple!

It will certainly take courage to make the appropriate decision but fundamentally I think it is because the technologist does not know who he is. What's more, he does not know what he wants to be. There is no objective! In fact, not only does he not have a final objective, but at no stage in any programme submitted has an expected outcome been defined.

No, I am wrong! In the second course described, the objective was declared to be 'to produce a laboratory worker who has a sound theoretical knowledge of medical technology and the associated practical expertise to enable him to carry out all the prescribed procedures'. However, this statement was immediately negated by stating that any higher level of attainment would be laid down in detail in the training programme.

This is a bit like trying to design a vehicle without having made up one's mind whether it will be a motorcar or a boat. Having decided what vehicle you want, you must decide then what you want it to do.

All the courses described so far have been ideas. And I use the word advisedly. An idea has been recommended and attempts have been made to fit the construction to various syllabuses but on no occasion has any attempt been made to establish a curriculum. Undoubtedly many of you are already asking—'What is the difference?'

A curriculum:

1. Is the plan of activities by which a student may learn to develop those critical skills necessary to achieve the stated aims of a particular course or subject.
2. It is designed for use by students, teaching staff and assessors (or users if you like) so that all will know what is to be achieved.
3. In planning such a curriculum it is important to remember that the material which a student is expected to learn must always relate to the final expected outcome of the course as a whole.

It must be evident therefore that one of the first things to be decided is the 'expected outcomes'. It is equally evident that because of the nature of the work required to be done in the laboratory these outcomes must involve some:

(a) creative ability,
(b) must generate logic and judgment and, above all,
(c) the achiever must be able to discriminate.

It is important therefore that when designing expected outcomes verbs such as 'understands', 'applies', 'evaluates', 'controls', 'identifies' and so on should always be kept in mind.

Several governing points are essential in the design of a curriculum:

1. The outcomes from a curriculum must be specified and measurable.
2. A curriculum should be suitable to the practical minimal needs of the various participants, whether they be society, student, faculty or institute or the profession.
3. The results of the curriculum must be achievable and must therefore take into account the student preadmission qualifications plus the human and physical resources.

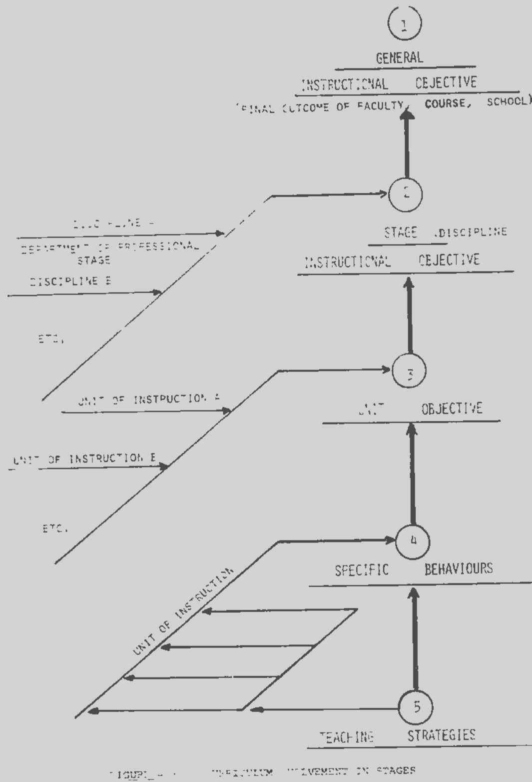


FIGURE 4 CURRICULUM EVOLVEMENT IN STAGES

4. Because the needs already mentioned must invariably change (if there is to be progress) the curriculum must be capable of being modified according to requirements.

Displayed in Figure 4 is the evolvement of such a curriculum design outlaid in stages. For clarity and, I hope, simplicity the chart is laid out in skeleton fashion only.

The most difficult decision of all is undoubtedly deciding on a general instructional objective; what is the final outcome desired or, more simply, what is wanted out at the end?

Having decided this some of the disciplines or subjects whether they be within departments or even sub-professions, will become obvious. Participation in any one of these however, must have an objective, an outcome or level of achievement must be decided for each and, as in each stage, this must relate to the final outcome.

To achieve this instructional objective the discipline or subject which for practical reasons, if for no other, will be divided into various units of instruction, but the make-up and construction of these units can only really be decided if collective unit objectives are clearly established first.

When particular teaching strategies are applied successfully to a unit of instruction specific behaviours or competencies will result. The teaching strategies might be the straightforward lecture, or the group discussion, laboratory application, assignments or field experience. The behaviour may be recognised as understanding, being able to apply, evaluate, identify or control, to name but a few.

What is described here is by necessity but a brief outline of curriculum development.

I would now like to put forward one final proposal for a course of study for your consideration which is governed by the criteria just outlined.

The proposal was discussed briefly by the Education Sub-Committee of the Medical Technology Board and can, I think, be said to utilise various points from the previous proposals where they are seen as capable of achieving specific objectives. They aim to attain at its end as an ultimate outcome, a capable person who typifies medical technology in its most advanced and progressive form.

Three objectives A, B and C are conceived in this proposal (see Fig. 5):

The overall or final instructional objective or outcome—

(C) A laboratory officer who applies the scientific methods of a selected (specialist) discipline and who develops, modifies, interprets and controls the quality of these methods for clinical diagnostic purposes.

The central or core objective—

(B) A medical technologist who undertakes responsibility for a unit, section or department of a clinical laboratory or small laboratory and who performs by request such procedures as will maintain an efficient, broad laboratory service for the medical care of the patient.

Both of these develop from personnel who appear first in the laboratory as—

(A) The laboratory trainee who uses his education programme productively and progressively in a clinical laboratory.

The behaviours which might be expected to gain objective A would be that the trainee:

1. Understands the basic sciences of chemistry, medical biology and laboratory technology.
2. Relates and applies these fundamentals to simple clinical biochemistry, medical micro-

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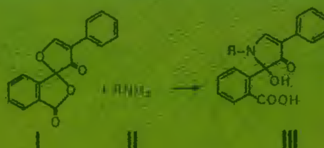

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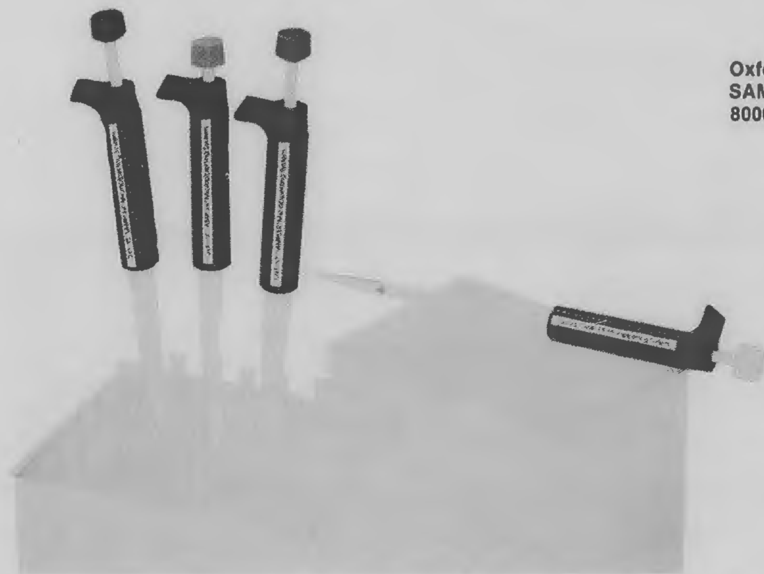

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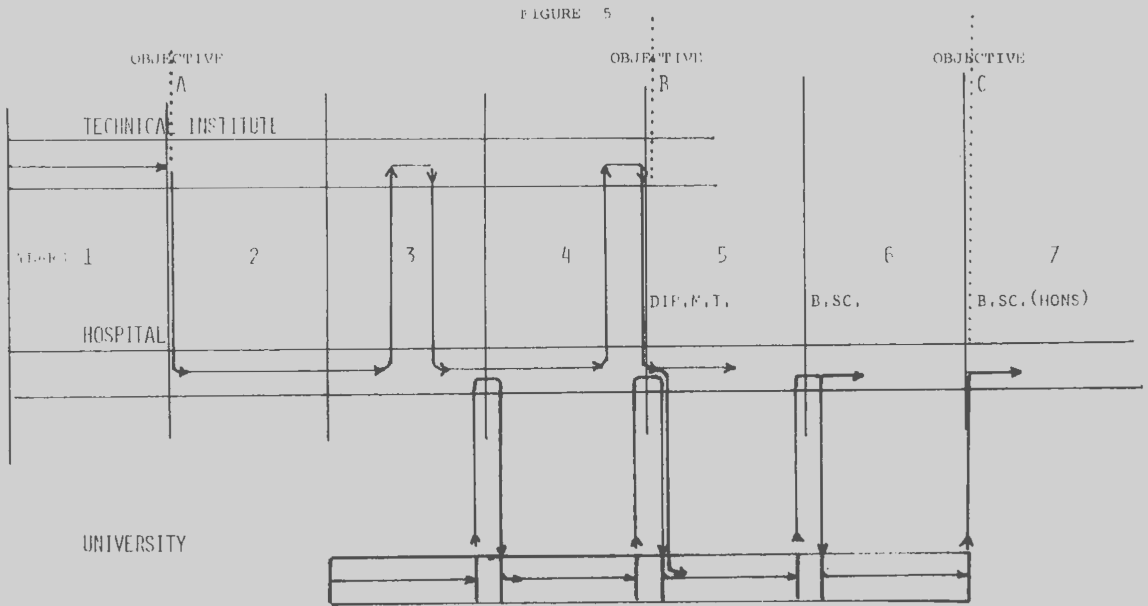

PROPOSAL 4 : (⟶) indicates pathways followed by a student to reach three objectives in an educational program for Medical Technology.

An alternate pathway through a University is also available when appropriate.

biology, histology and cell morphology, haematology and blood grouping procedures.

3. Uses and understands the terminology of these disciplines.
4. Operates with confidence simple equipment for these procedures.
5. Recognises and applies control measures to the procedures used.
6. Questions the outcome of the procedures performed.

Many units of instruction will immediately become evident, each one in turn having as its objective the production of the specific behaviours just described which would allow appropriate application immediately within clinical practice. The time required to achieve this level I see as being one year (see Fig. 5).

To attain the next objective (B) further competencies would need to be demonstrated. These I see as being:

1. Understands basic departmental and staff management methods.
2. Uses appropriate scientific procedures for estimating and supplying clinically diagnostic data.
3. Understands the theoretical background of core laboratory disciplines.
4. Applies the background to new developments.
5. Evaluates the findings from the scientific procedures for clinical situations.
6. Controls the quality of the scientific procedures.

Obviously a longer period of clinical application is necessary here and a period of three years is recommended.

Equally obvious is the need for appropriate theoretical support and I would suggest that within this three years a minimum of at least two sandwich courses, consisting of 12 weeks, be undertaken at times of choice and convenience to the laboratory and to the educational centre. These sandwich courses to supply the academic material necessary for the understanding of a broad spectrum of laboratory knowledge.

You will see that I have labelled this level as being worthy of a Diploma in Medical Technology.

You will also see that I have postulated that the level should academically equate with that achieved at the end of a second year of a university science degree and see it as imperative that communication and liaison be such that this degree of reciprocity is assured.

Opportunity for selection by merit, supported by sponsorship or scholarship should then be available for the appropriate diplomate to complete a specifically designed science degree or even, should that diplomate justify it, proceed to an honours level. The honours level being accomplished by the selection and study of a specialist area within the clinical laboratory diagnostic service.

At no time should a candidate for this stage of such a programme be divorced from the clinical application and background and each should be sandwiched with the other as is appropriate.

Such a curriculum development plan or course needs greater detail. But, once an understanding is gained of what is to be achieved and at which stage, each section can be taken separately and by being subjected to the same approach evolved into a detailed plan of action worthy of implementing.

One of the other proposals may appeal but I would recommend the last, the ultimate outcome of which, and indeed the outcomes of each stage, will produce a spectrum of Medical Technology capable of meeting any diagnostic demands.

The Laboratory Diagnosis of Alpha$_1$-Antitrypsin Deficiency

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Clinical Biochemistry Department, Christchurch Hospital, Christchurch

Received for publication, June 1974

Summary

A severe deficiency of alpha$_1$-antitrypsin predisposes to premature emphysema and liver disease. An absent or markedly reduced α_1 band on visual inspection of a serum protein electrophoresis strip suggests this diagnosis. It should be confirmed by specific measurement of alpha$_1$-antitrypsin by radial immunodiffusion. Phenotyping of alpha$_1$-antitrypsin is described. This will reveal other genetic variants of less clinical importance.

Introduction

Alpha$_1$-antitrypsin, a glycoprotein of molecular weight 53,000, is the main component of the α_1 globulin band on serum protein electrophoresis. Like other serum proteins, except the immunoglobulins, it is synthesised in the liver and secreted into the bloodstream. It has a normal serum level of 200-500 mg/100 ml, although this value increases during tissue destruction, inflammation, neoplasia, pregnancy and oestrogen containing oral contraceptive therapy. Alpha$_1$-antitrypsin is the major serum antiprotease and accounts for approximately 90 percent of the serum antitryptic activity (Eriksson, 1965)[2].

Like all proteins, the synthesis of alpha$_1$-antitrypsin is under genetic control, and variants are thus likely. Since 1963 when the first genetic deficiency of alpha$_1$-antitrypsin was described by Laurell and Eriksson (1963)[9], twenty-one electrophoretic variants have been found (Kueppers 1973)[8]. While many of these variants are of no clinical significance, some cause a low serum alpha$_1$-antitrypsin level which is of clinical importance.

In 1967 Fagerhol and Laurell (1967)[5] proposed the protease inhibitor (Pi) system. This system interprets the electrophoretic variants as being due to codominant alleles at a single autosomal locus (called Pi). Each allele is assigned a letter (E, F, G, I, M, P, S, R, V, W, X, Z) which depends upon the electrophoretic mobility of the variant. The normal allele is PiM and the normal phenotype is MM, which is present in approximately 90 percent of Northern and Central Europeans. However, the frequency of the MM phenotype varies from 70 percent (in Portuguese) to 100 percent (in Mongoloids) (Fagerhol, 1972)[3]. The S and Z variants are the next most common alleles. In an unpublished survey of New Zealand Europeans carried out in this laboratory, we found 88 percent were MM, 7 percent MS, 4 percent MZ and 1 percent were other phenotypes.

The presence of either or both of the Pi^s and Pi^z alleles results in a lowered level of serum alpha$_1$-antitrypsin. Thus as MM has a

normal level (100 percent), an MS 70 percent, an MZ 60 percent, an SS 60 percent, an SZ 30 percent and a ZZ 15 percent of the normal level. However, in practice there is considerable overlap of the values for the different phenotypes. This is probably due in part to the wide normal range (200-500 mg/ 100 ml).

Low levels of serum alpha$_1$-antitrypsin, especially seen in ZZ and SZ phenotypes, predispose the early onset of the lung disease emphysema. Typically ZZ individuals will develop emphysema and the resulting shortness of breath in their twenties or thirties (Lieberman, 1973)[10]. The occasional ZZ individuals in their sixties or older who have only mild emphysema are non-smokers and have worked in an environment free of lung irritants. The MZ phenotype also predisposes to emphysema, but not to the same extent.

The presence of the Pi^z allele is also related to liver disease in both infants and adults (Sharp et al., 1969)[12]. It appears that the Z allelic product is abnormal and cannot be secreted from the liver (Bell and Carrell, 1973)[1]. This causes a build up of abnormal alpha$_1$-antitrypsin in granules in the liver. These deposits may be seen in PAS stained liver sections (pretreated with diastase to remove glycogen) (Lieberman *et al.*, 1972)[11].

The clinical aspects of alpha$_1$-antitrypsin deficiency has been recently reviewed by Janus and Carrell (1974)[4], Kueppers (1973)[8], Lieberman (1973)[10] and Greenberg *et al.* (1973)[6].

Methods and Results

Serum Protein Electrophoresis

On routine protein electrophoresis (Fig. 1) the severe deficiency states (phenotypes SZ and ZZ) of alpha$_1$-antitrypsin may be detected by a markedly reduced or absent α_1-globulin band. All such cases should be checked by radial immunodiffusion or antitryptic activity measurements. As a gross alpha$_1$-antitrypsin deficiency is present in approximately 1 in 750 of the population, and in a considerably higher proportion of respiratory and liver disease patients, a low α_1 level is probably not too uncommon. Visual inspection of a long electrophoretic run is the best way to detect this. The abnormality may often be missed if only a scan is performed. In our experience cellulose acetate or cellogel is the most satisfactory supporting medium for screening purposes.

The alpha$_1$-antitrypsin variants causing a partial deficiency are very difficult if not impossible to detect with any certainty by serum protein electrophoresis. As these patients are not at the same risk as those severely deficient in alpha$_1$-antitrypsin, it is probably not so important at the present time to detect partial deficiencies.

FIG. 1.—Serum protein electrophoresis (cellulose acetate) on individuals with normal (1, 2, 3, and 11), intermediate (D.C. and 12) and low levels of α_1 antitrypsin.

Radial Immunodiffusion

The level of serum alpha$_1$-antitrypsin may be determined by radial immunodiffusion. Suitable plates are commercially available. We used M-Partigen plates supplied by Behringwerke. A 1 in 10 dilution of serum is used if the α_1 band is normal on serum protein electrophoresis, a 1 in 5 dilution if the α_1 band is of intermediate intensity and undiluted serum

Table I
Serum alpha$_1$-antitrypsin levels as determined by radial immunodiffusion for various phenotypes. Phenotyping was done by acid starch gel electrophoresis.

Phenotype	MM	MZ	MS	SS	SZ	ZZ
Individual Values in mg/100 ml	290	165	220	120	90	84
	260	165	185	185	90	30
	235	180	175	145	60	37
	360	170	230	165	66	41
	340	185	175		100	43
	380	180	135		95	42
	475	135	185		99	68
	520	175	240		85	64
	225	140	230		75	81
	320	125	165		85	27
	275	125	200			40
	225	175	175			59
	235	130	200			29
	220	175	210			29
	235	165				25
	215	175				22
	205	185				25
	225	165				31
	290	130				
	600	135				
	235	170				
	300	160				
	195	165				
	270	120				
	335	160				
	230	135				
Average	296	158	195	154	85	43
Range	195–600	120–180	135–240	120–185	60–100	22–84
% Normal Value	100	54	66	52	29	15

if there appears to be an almost complete absence of the α_1 band. Serum levels of less than 100 mg/ 100 ml are a good indicator of a severe alpha$_1$-antitrypsin deficiency, and levels from 100-200 mg/100 ml indicate a partial deficiency. Levels over 200 mg/ 100 ml are probably indicative of a normal phenotype (MM), but other phenotypes may have levels in this range (see Table I). This is particularly so for women with a partial deficiency who are pregnant or on oral contraceptives. This also applies to any person with a partial deficiency who has an acute infection. The effect of oestrogens and acute infection is to raise intermediate levels into the low normal range.

Serum Antitryptic Activity

Serum antitryptic activity may be determined by the method of Eriksson (1965)[2]. While alpha$_1$-antitrypsin is not the only antitryptic protease in serum it is the main one and it has been shown that antitryptic activity is a good indicator of alpha$_1$-antitrypsin levels. Serum antitryptic activity measurements will give similar results to radial immunodiffusion and for the clinical laboratory without a special interest in alpha$_1$-antitrypsin, it would be sufficent to measure alpha$_1$-antitrypsin by radial immunodiffusion or the serum antitryptic activity.

Acid Starch Gel Electrophoresis

Acid starch gel electrophoresis was introduced by Fagerhol and Braend (1965)[4] and is the basis of the protease inhibitor phenotyping system. The method is described in some detail below.

Materials

Gel buffer—21 g citric acid, 23 g tris in one litre. pH 4.9.

Gel—33 g hydrolysed starch (Connaught). 17.5 ml gel buffer and 250 ml distilled water was heated and evacuated and poured into a gel plate measuring 20 cm × 12 cm × 0.8 cm. The gel was allowed to solidify by placing it at 4°C for one hour.

Anode buffer—23.5 g citric acid, 30.4 g K_2HPO_4 in two litres. pH was 4.4.

Cathode buffer—74.2 g boric acid, 10 g sodium hydroxide in two litres. pH was 7.9 to 8.0.

Fixing solution—acetic acid/methanol/water in ratio of 1:5:5 by volume.

Staining solution—1 g amido black (naphthalene black 12B) in one litre of fixing solution.

Method

Small filter paper (Whatman 3MM) wicks (approximately $1\frac{1}{2}$-2 cm × $\frac{1}{2}$-$\frac{3}{4}$ cm) were soaked in serum and then inserted in a cut in the gel approximately 2 cm from a long edge. Ten samples were inserted into each gel. The loaded gel was placed in a water-cooled electrophoresis tank with the samples at the cathode end and 3MM filter paper used as wicks. The gel was run for four hours at approximately 45 mA (constant current) with a voltage of 100-160 v.

It is possible to run two gels in parallel for eight hours at approximately 48 mA and 80-100 v.

After the run the wicks were removed and the gel sliced. Staining solution was poured on the gel, left for 10 minutes, and then the gel was fixed and cleared in the fixing solution. The alpha$_1$-antitrypsin (or pre-albumin) bands were clearly visible within an hour.

Results and Interpretation

Figure 2 below is a schematic diagram of the patterns observed for various phenotypes. Compare with Figure 3, a photograph of an actual gel. Interpretation of the patterns seen upon

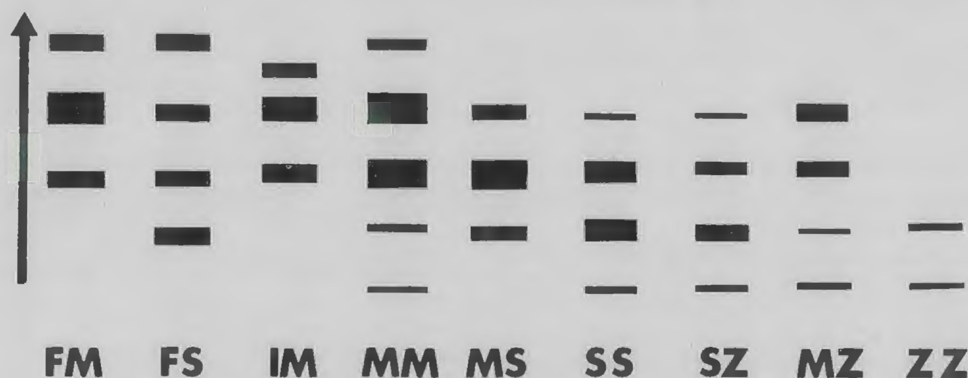

FIG. 2.—Schematic diagram of acid starch gel electrophoretic patterns for various phenotypes of α_1 antitrypsin.

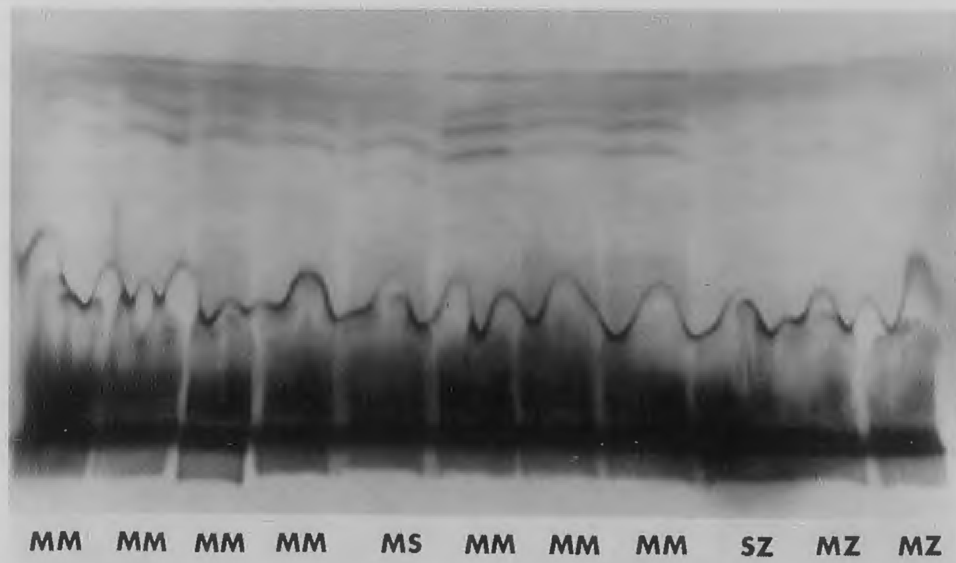

FIG. 3.—Photograph of a gel showing the MM, MZ, ZZ, MS, SS and SZ phenotype patterns.

acid starch gel electrophoresis requires considerable experience as the differences between some phenotypes is rather subtle, depending on both the intensity and distribution of the bands.

The MS, FM, IM and FS phenotypes are relatively easily identified. The two main problems are in distinguishing the MM and MZ phenotypes and in typing other rare phenotypes, particularly SS, SZ, and ZZ. The main difference between the MM and MZ phenotypes is one of intensity of bands. While the MM and MZ phenotypes can usually be distinguished, radial immunodiffusion or serum antitryptic activity measurements are useful in doubtful cases. Especially in patients who are pregnant, on oral contraceptives, or with acute inflammation, elevated alpha$_1$-antitrypsin levels occur and it may occasionally be very difficult to definitively identify an MZ as such.

The rare phenotypes, notably SS, SZ and ZZ are, with experience, not too difficult to identify, especially if radial immunodiffusion or serum antitryptic levels are available.

Two Dimensional Immunoelectrophoresis

This technique developed by Fagerhol and Laurell (1967)[5] is a sophisticated extension of the acid starch gel electrophoresis method and is mainly useful in confirming the MZ phenotype in difficult cases.

Materials and Methods

Barbitone buffer—9.2 g barbituric acid, 51.5 g sodium barbitone in five litres. pH 8.6.

0.3 g agarose (Baker) and 15 ml of barbitone buffer were heated in a water bath until the agarose had completely dissolved. 6 ml of clear agarose solution and 200 μl of rabbit immunoglobulin against human alpha$_1$-antitrypsin (Dakopatts) were mixed and poured onto a preheated and clean, warm glass plate (approximately 7½ cm × 5 cm). The solution solidified into a gel, which was left at least half an hour before use.

Approximately 1 cm from a long edge, a trough of approximately 5 cm × ½ cm was cut into the agarose gel. Into this trough was placed a strip from an unstained starch gel which had been run as previously described. The starch gel strip had only one sample on it and had been cut out in the direction of electrophoresis. The alpha$_1$-antitrypsin bands were near the middle of the strip, and the solvent front was ½-1 cm from one end of the strip.

Two samples on their respective gel plates were placed side by side in an electrophoresis tank with the samples at the cathode end. Filter paper (Whatmann 3MM) was used as wicks. The plates were run for 3-3½ hours at 300-320 v and 20-30 mA.

The plates were removed from the electrophoresis tank and placed in saline solution for 24 hours or longer. They were then stained with 0.1 percent amido black in fixing solution for 10 minutes and washed in acetic acid/methanol/water (1:5:5).

Results and Interpretation

Adequate results from two-dimensional immunoelectrophoresis are mainly dependent on a good separation of bands upon acid starch gel electrophoresis. This is to be expected as this crossed electrophoresis is really only a 'scan' of the alpha$_1$-antitrypsin on the first dimension gel. Hence, the two-dimensional immunoelectrophoresis seldom gives more information than a good result upon acid starch gel electrophoresis. However, it may help distinguish MZ variants with a high alpha$_1$-antitrypsin level from the MM phenotype because of the presence of slightly larger Z bands. This is best seen by studying Figure 4 which is a schematic diagram of various phenotypes on two-dimensional immunoelectrophoresis. Photographs of actual patterns on agarose gels are shown in Figure 5.

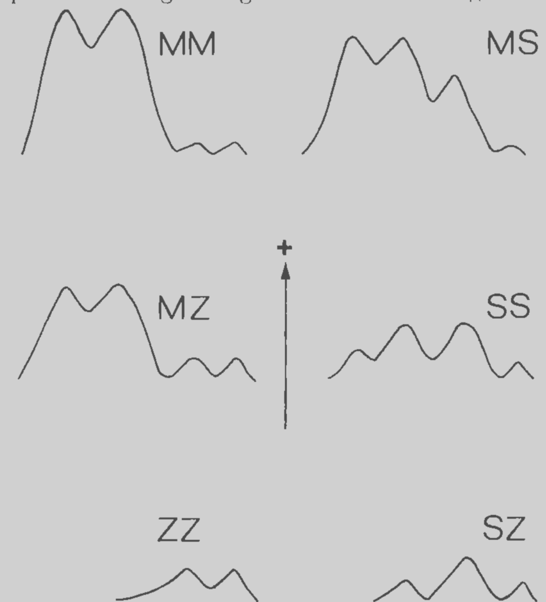

FIG. 4.—Schematic diagram of results obtained by two-dimensional immunoelectrophoresis for various phenotypes. Note the higher and wider Z bands in the MZ compared with the MM.

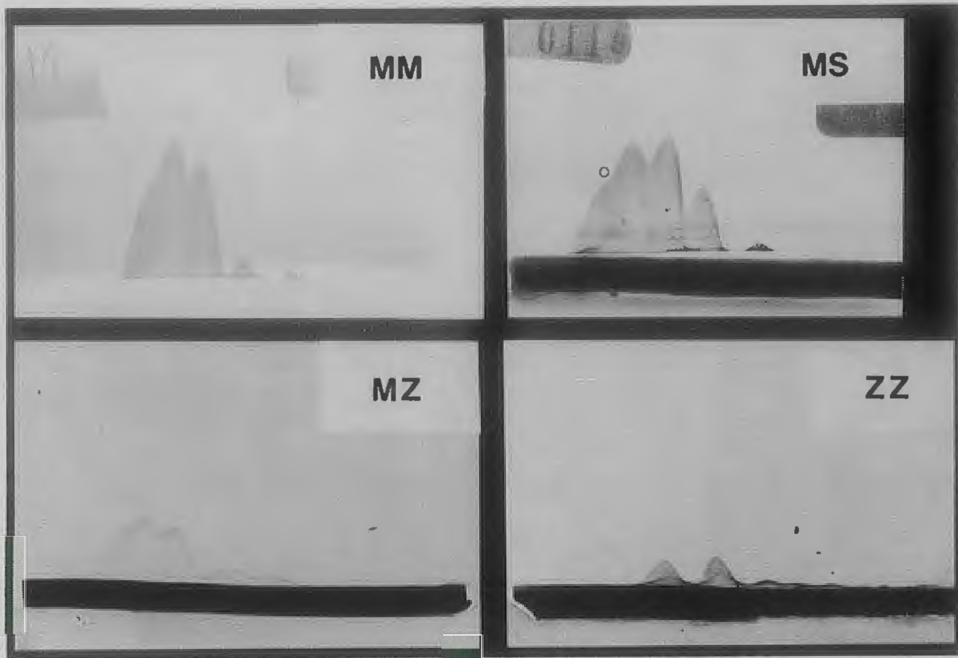

FIG. 5.—Photographs of MM, MZ, MS and ZZ phenotypes on two-dimensional immunoelectrophoresis.

Discussion

While most clinical laboratories will not have to consider doing alpha$_1$-antitrypsin phenotyping, all should be aware of the significance of alpha$_1$-antitrypsin deficiency. Particularly important at this time is the detection of individuals with a severe deficiency. While careful visual inspection of the α_1 band on serum protein electrophoresis strips will detect these severe deficiencies, radial immunodiffusion values or serum antitryptic activity levels are necessary to confirm the severe deficiency.

At the present time, alpha$_1$-antitrypsin deficiencies, like all genetic disease, cannot be cured. However, early detection of the severe deficiency individuals who are at grave risk of developing crippling respiratory disease or liver disease is most important. It allows them to be counselled on ways to protect their lungs (e.g., not smoking and avoidance of dusty environments). In this way the person with severe alpha$_1$-antitrypsin deficiency may delay the onset of the inevitable emphysema to their sixties and lessen its ultimate severity.

Acknowledgments

We would like to thank Associate Professor R. W. Carrell for his continuing encouragement and interest in this work. P.R.J. was supported by the Canterbury Medical Research Foundation. This project also received support from the Medical Research Council of New Zealand.

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Assessment of Ion Exchange Chromatographic Separation of Creatine Phosphokinase Isoenzymes as a Basis for Screening for Malignant Hyperthermia

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Middlemore Hospital, Auckland

Adapted from a thesis submitted in partial fulfilment of the examination for Qualified Technical Officer, 1973, and summarised by D. A. McArthur

Introduction

Malignant hyperthermia is a recently noted but often fatal complication of general anaesthesia. After the administration of any commonly used muscle relaxants the susceptible patient develops a rapid rise in temperature and muscle rigidity; temperatures often exceed 106°F with a mortality rate of 70-80 percent[8]. Muscle rigidity, metabolic acidosis, hyperkalaemia, release of potassium, myoglobin and other cellular components into the blood stream occurs, and this is attributable to alteration in muscle cell permeability and, or necrosis of muscle. The susceptibility to develop malignant hyperthermia is due to a genetic anomaly[5], mostly in individuals with a myopathy which is inherited as a dominant characteristic, and also in young patients in whom physical abnormalities are present.

Middlemore Hospital has a plastic surgery and orthopaedic ward for children with congenital deformities and as these children may be anaesthetised several times we have a special interest in detecting malignant cases prior to operations.

Currently a raised serum Creatine Phosphokinase level appears to be the best predictive index routinely available. Some investigation of the place of serum pyrophosphate[7, 9] analyses has been carried out but correlation between this parameter and malignant hyperthermia is not good. Clinical appraisal is important, with patients showing congenital muscular defects being considered in a raised risk category.

As serum Creatine Phosphokinase activity derives from various tissues and is therefore relatively non-specific, being raised in various conditions, this limits the usefulness of the test. It was therefore decided to investigate a possible method of separation of CPK isoenzymes by using an Ion-exchange Resin to separate the protein fractions and to perform CPK enzyme estimations on the protein eluant. It was hoped that relative increases of various isoenzymes would provide a screen for potential risk of malignant hyperthermia.

Ion-exchange chromatography is a relatively new type of absorption chromatography which may be loosely defined as the analysis of mixtures of compounds by differential absorption on a solid support medium through which the mixture passes. Ion-exchange chromatography is typically performed down a vertically mounted cylindrical column. Simply, a sample of the mixture to be fractionated is applied to the top of the tube packed with particles of insoluble material containing chemically bound charged groups with counter ions capable of reversible exchange. The particles interact electrostatically with compounds in the sample. A liquid phase is passed down the column and the compounds are carried through at different rates depending on their interaction with the particles. Hence, the rate of migration of a compound depends on the relative numbers of its molecules that remain in the liquid phase, compared with the number that are electrostatically bonded to the ion-exchange particles at every point down the column.

Ideally, migration of the compounds of interest will be appreciably slower than the flow of liquid phase through the column so that multistage absorption and desorption of the compounds is achieved.

In the study presented, a survey of normal sera was compared with results from patients with raised CPK values due to heart muscle defects and also from families of known cases of malignant hyperthermia to establish any abnormal pattern of CPK isoenzymes which was specific to either group. As an ultimate aim it was hoped that information obtained would lead to the development of a simplified method suitable for use in screening for potentially dangerous cases prior to operations.

It is of interest to note that the paper of Zsigmond and associates reported that in contrast to the predominance of the MM isoenzyme (slow moving) in normal adult serum and skeletal muscle, a dominant BB isoenzyme (fast moving) was found in the affected malignant hyperthermia patients and their families.

Method

(1) Ion Exchange Chromatography.

After considerable investigation of resin, serum dilution, molarity gradient, rate of elution, the following methodology was adopted.

Whatman DE 32 ion-exchange resin was used in a glass column, diameter, 2.5 cm. The column height was 6.2 cm.

After rinsing the column with 0.05 M Tris-HCl buffer, pH 9. A serum/buffer dilution (1 ml serum diluted to 40 ml with 0.005 M Tris-HCl buffer was allowed to penetrate resin column. Molarity gradient of buffer in reservoir was increased from 0.05 M by addition of 1 M NaCl from burette.

Eluant was collected at approximately 2 drops/sec after passing through a flow cell in the recording spectrophotometer (Unicam SP800).

Absorbance/time scan of protein was read at 295 nm and the eluant was collected in approximately 2.3 ml fractions of estimation of CPK levels.

(2) Enzyme Estimation

- (i) 2.0 ml eluant.
- (ii) 0.03 ml 10 percent v/v acetic acid.
- (iii) 0.2 ml CPK reagent (Rosalki reaction).

It was found convenient to prepare a concentrated kitset reagent with only one-third of the stipulated volume of water.

- (iv) Solution mixed and incubated 5 mins at 37°C.
- (v) Initial absorbance (A_5) then read at 340 nm against reagent blank as reference.
- (vi) After incubation for 1 hour at 37°C, final absorbance read (A_{60}).
- (vii) Enzyme level $= A_{60} - A_5$.

The results were presented graphically by calculating the molarity at various stages of the chromatograms and plotted the following parameters against time.

- (i) Protein concentration (absorbance at 295 mm).
- (ii) CPK enzyme level ($\triangle A$).
- (iii) Molarity of buffer.

Six normal sera, six abnormal (high CPK) sera and four sera from cases of families associated with malignant hyperthermia were assessed on the basis of the above procedure. The following are typical graphs illustrating the three panels (Fig. 1).

- (a) Normal.
- (b) Raised CPK (heart or muscle).
- (c) Malignant hyperthermia.

For comparison, in this summary protein concentration and CPK isoenzyme level have been graphed against molarity of buffer.

Discussion

By comparison of results from the normal, abnormal, and malignant hyperthermia surveys, there was a definite increase of the CPK isoenzyme activity in abnormal and malignant hyperthermia patients, which had already been established in previous work.

As there were only a relatively few known cases of malignant hyperthermia from which we could obtain a macro specimen to analyse, the scope of comparison was limited. However, from the four malignant hyperthermia cases in the Auckland to Hamilton area available, there did not appear to be any specific enzyme pattern common to all four specimens tested.

In each normal serum tested most of the major enzyme peak was eluted by buffer molarity of 0.16. In five out of the six abnormal sera only approximately 50 percent of the enzyme was eluted by buffer molarity of 0.16 with the remainder of the major peak being eluted by buffer molarity of 0.21. The malignant hyperthermia sera did not fit as a group, into this latter division.

As mentioned in the introduction the recent paper by Zsigmond and associates[10] reported that in contrast to the predominance of MM isoenzyme (slow-moving) in normal adult serum and skeletal muscle a dominant BB isoenzyme (fast-moving) was found in malignant hyperthermia patients and their families.

In the studies here presented the major enzyme fraction is found in the gamma globulin protein region and is presumably MM isoenzyme. This was found in each of the four sera from families associated with hyperthermia.

In one of these four sera however this band was much finer and smaller and was followed by a large band on increasing the buffer molarity. This second band may have been predominantly BB isoenzyme.

The small panel of sera from families associated with malignant hyperthermia did not yield a specific pattern by this technique. Two sera of this panel presented patterns typical of those from normal sera. As the total CPK in these two cases was within normal limits it is likely that these persons were not exhibiting an 'at-risk' characteristic. On the other hand

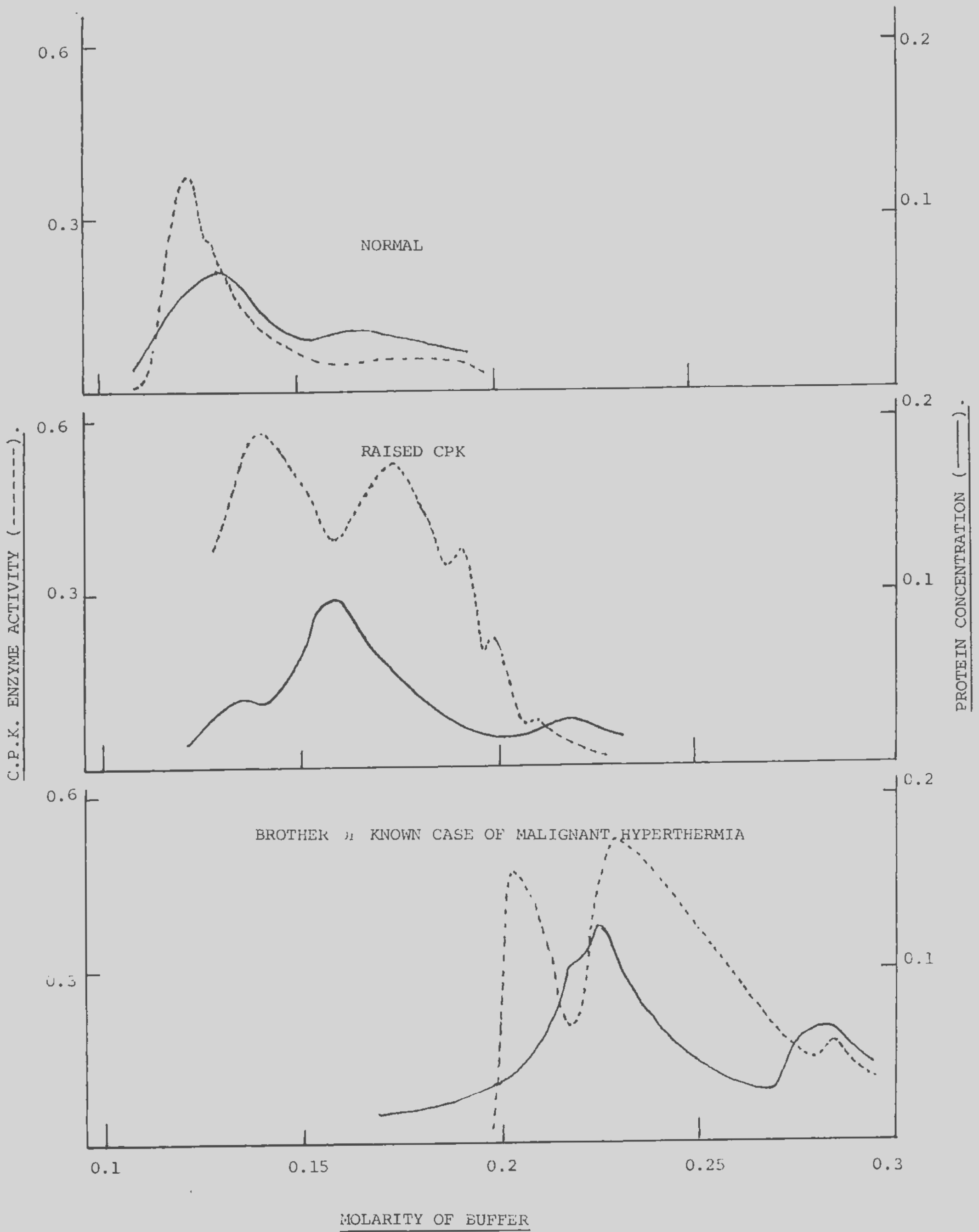

FIG. 1.—Representative examples of separation of CPK isoenzymes.

the serum with the highest total CPK from this panel presented a CPK pattern greatly different from others in the survey.

In this case elution of the initial enzyme peak was completed when the buffer molarity reached 0.22. With ascending buffer molarity a further large peak was eluted over the molarity range, of 0.22 to 0.29.

On the basis of this limited survey it would appear that simplified screening test for hyperthermia 'at-risk' patients presenting for surgery may be developed using such ion-exchange media with specific investigation of elution by buffer of molarity 0.22 followed by elution by buffer of molarity 0.29.

It was clear that further investigation of sera from cases of malignant hyperthermia would be required to confirm this potential approach to pre-operation screening.

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Serum Lipid Techniques in New Zealand

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Based on a paper delivered to the NZIMLT Annual Conference, New Plymouth, August 1974

Introduction

Ever since Frederickson introduced his classification of the hyperlipoproteinaemias in 1965, an increasing number of laboratories estimate the various lipid fractions. Also since then new techniques, interpretation of results and screening tests have been introduced. This report will present the results of a lipid questionnaire sent to biochemistry departments of pathology laboratories in New Zealand.

Results

Questionnaires were sent to forty-four public hospital laboratories and twenty-one private laboratories. Forty public hospital laboratories and nineteen private laboratories replied, of these eight laboratories do not estimate any lipid fraction and send them to other laboratories for analysis. The questions, and their results will be presented individually.

Question 1. Do you phenotype according to Frederickson?

Thirty laboratories do, twenty-one do not.

Question 2. What method do you use for serum cholesterol?

For simplicity the methods were grouped together under the main methodologies, although different extraction reagents and many modifications to the techniques were used.

AUTOMATED

Ferric Chloride/Sulphuric Acid: 4
Acetic Anhydride/Sulphuric Acid: 7

NON-AUTOMATED

Ferric Chloride/Sulphuric Acid: 23
Acetic Anhydride/Sulphuric Acid: 17

Eight laboratories used commercial kitsets.

Question 3. What method do you use for serum triglycerides?

Again for simplicity methods were grouped together under the main methodologies.

AUTOMATED

Fluorimetric Hantzch: 4
Colorimetric Hantzch: 2

NON-AUTOMATED

Fluorimetric Hantzch: 1
Colorimetric Hantzch: 33
Enzymatic (U.V.): 3

Twenty-five laboratories used commercial kitsets.

Question 4. What method do you use for serum total lipids?

* Present address: **Biochemistry Department, Memorial Hospital, Hastings.**

Fifteen laboratories estimated total lipids and all used the sulphuric-phosphovanillin reaction. Of these, three laboratories used commercial kitsets. All these laboratories were asked the additional following questions.

(a) Why does your laboratory estimate serum total lipids?

(b) What use do you think serum total lipids have in a lipid screening profile?

(a) The main reasons stated for estimating serum total lipids are:

1. The doctor specifically requests it.
2. To quantitate the lipoprotein fractions from the electrophoretogram.
3. As a rough check on the cholesterol and triglycerides results.

(b) For the latter question the often expressed opinion was that serum total lipids do not seem to have any value in lipid screening profiles.

Question 5. What support medium, buffer and stain do you use for lipoprotein electrophoresis?

SUPPORT MEDIUM	
Cellulose Acetate:	32
Agarose Gel:	3

BUFFER	
Tris/Barbital:	22
Barbitone/Sodium Barbitone:	11
Barbitone/Acetate:	1
Barbitone/EDTA:	1

STAIN	
Oil Red 0:	26
Sudan Black 10 B:	1
Amido Black 10 B:	1
Fat Red 7 B:	3
Sudan 1 V:	3
Schiff's stain:	1

One laboratory uses acrylamide gel on selected cases.

Question 6. Do you use any screening tests?

Three laboratories do and all used Precipitest (Searle Diagnostics) as a preliminary screening test.

Question 7. Do you estimate any of the other lipid fractions?

One laboratory performs immunological studies on selected cases using anti-Apo A. B and C and anti L P X.

One laboratory estimates phospholipids on selected cases.

One laboratory estimates alpha-lipoprotein cholesterol using heparin-$MnCl_2$ precipitation on selected cases.

Five laboratories use a commercial kitset for the estimation of serum low density lipoproteins.

Question 8. Do you perform lipoprotein electrophoresis on all samples submitted for Fredrickson's classification?

Ten laboratories do so. Laboratories not doing so either have no facilities for lipoprotein electrophoresis, or only do so when serum cholesterol and/or triglycerides are elevated.

Question 9. In your cholesterol method do you correct for bilirubin interference? (Only laboratories using the direct non-automated Liebermann-Burchard reaction.)

Two laboratories do so and both subtract 5 mg cholesterol per mg of bilirubin. Of the fifteen laboratories who do not correct, three include a serum blank.

Question 10. Do you scan your electrophoresis strips?

Fourteen laboratories do so, and of these four report the lipoprotein fractions in mg/100 ml as lipid.

Question 11. Do you subtype Fredrickson's type II?

Sixteen laboratories do and twenty do not. Of the latter, five laboratories leave the interpretation of the results over to the medical staff.

Question 12. How many times do you do lipid analysis on a patient before reporting a definite phenotype?

At the clinician's discretion:	17
Three times:	8
Twice:	4
Once:	5

Question 13. What instructions do you give the patient before taking blood for lipid analysis?

All fifty-one laboratories asked them to fast overnight. Additionally, four laboratories stipluated a two-week normal diet; three laboratories stipulated two days alcohol abstinence. Two laboratories stipulated three days alcohol abstinence plus two weeks normal diet; two laboratories stipulated a three-day normal diet: and one laboratory stipulates a four-day low fat diet.

One laboratory does not use a tourniquet when taking blood and another laboratory is studying the effects of posture on blood lipids.

Question 14. Do you use any other methods or formulas as aids when phenotyping the hyperlipoproteinaemias?

Four laboratories use the following formula for calculating the beta-lipoprotein cholesterol.

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PFIZER SAMPLER	613E	300 µl	yellow tips
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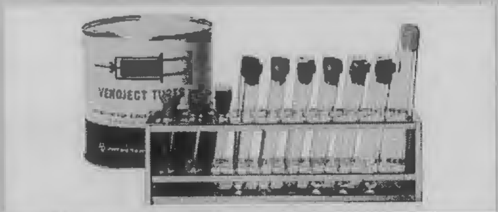

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Beta cholesterol = total cholesterol

$$- \left\{ \frac{\text{Triglycerides}}{5} + 40 \right\}$$

Results above 210 indicate type II, below 190 type IV.

Two laboratories perform ultra centrifugal analysis on suspected type III subjects.

Question 15. What is your laboratory's normal range (including age and sex differences) for cholesterol and triglycerides?

Most laboratories quote only one normal range, some quote normal ranges for two to three groups, while only a few quote a comprehensive normal range dependent on age. However some laboratories quoting one normal range on their report form make an allowance for age when phenotyping. Quite a few laboratories using commercial kitsets quote the normal range from the company's literature. Depending on method—the upper normal value for serum cholesterol was as far ranging as from 280 to 350 mg/100 ml: for serum triglycerides from 140 to 220 mg/100 ml and for serum total lipids from 750 to 1,000 mg/100 ml.

Summary

The results of a recent lipid questionnaire sent to New Zealand pathology laboratories regarding methodologies are presented. As the questionnaire was sent in October 1973, the above results may not be completely up to date as some laboratories may have changed or added to their methods.

Acknowledgments

The author wishes to thank Dr M. Lever and Mr I. Bardsley for helpful comments and encouragement in carrying out this survey. To Miss P. Andreoli and Miss M. Curry for clerical assistance and to Mr R. Chowdhury, Surgeon-Superintendent of Hawera Hospital, for permission to publish.

Finally thanks to all the participating laboratories without whom this survey would not have been possible.

A Reliable Method for Platelet Adhesion

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Received for publication, June 1974

A satisfactory method for measuring platelet retention in glass bead columns is difficult in terms of the ability to get the necessary components in this country. Therefore a description of the needed modification of the method of Bowie and associates[1, 2, 3] (which is an *in vitro* development from the method of Salzman[5]) to fit the local scene would be appropriate. The main item required for this method is a Harvard infusion pump, model 975 (Geo. Wilton), to provide a standard, constant rate of blood flow through the column.

To describe in detail the components:

1. *Plastic tubing,* 3 mm ID, 5 mm OD. We discovered, as did Bowie *et al.,*[3] that the type of plastic was critical. Earlier columns made by us from polyvinyl (Pharma) gave completely normal results with severe von Willebrand's disease. A spectral scan on the plasma from effluent blood revealed characteristic peaks of haemoglobin which confirmed the visual impression of slight haemolysis, leading to platelet aggregation from erythrocyte ADP, which caused the normal result. Other tubings which were similarly unsuitable were a variety of polyvinyls and tygon (Technicon). Silicone made no difference. Polyvinyl chloride (A. S. Patterson Ltd) was suitable, and polyethylene should be also, though this is not yet tested by us.

The tubing was rinsed out with tapwater, and dried on a vacuum pump. Overall column lengths were precut to 25.5 cm.

2. *Glass beads.* These were the biggest difficulty of all. Bowie's method (and many others) used Superbrite 070 beads (3 M Co) but these beads were unobtainable. After much trial, the closest beads to these for retention potential were Jencons ballotini No. 8 (Geo. Wilton). These are 0.45-0.5 mm OD and were washed and dried before use. Measurement of 2.62 g for column was conveniently done by filling a 2 ml pipette (heat sealed at the tip end) to a volumn of 1.6 ml, using a small funnel.

3. *Column construction.* The columns of Salzman and Bowie, using Becton-Dickinson adaptors were difficult to prepare, and too costly to discard. Prompted by a very simple

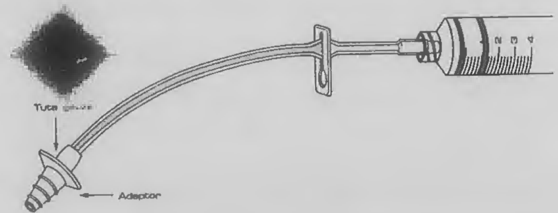
column (using soda straws) described by Zacharski and McIntyre[6], our current column can be fabricated in seconds (Fig. 1). A XYLON adaptor (Townson and Mercer) XT 2040 8 mm OD was used to seal the distal end of the column. These were rinsed and reused. To seal the beads at this end, a small square of filter gauze (Tuta Laboratories) as used in giving sets was inserted when press-fitting the adaptor on to the tubing. Beads from the pipette were poured in through a small funnel, and the column was tapped several times to pack the beads. At the proximal end there is a 1-1.5 cm gap, which was temporarily sealed using a keyhole clip from used taking sets.

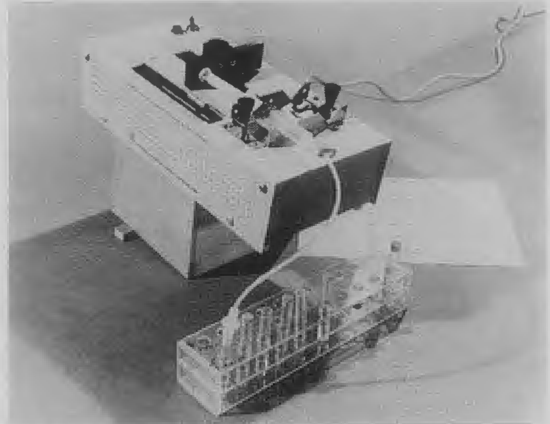
4. *Blood sample.* Approximately 0.1 ml of 500 u/ml heparin is placed in a Jintan 10 ml syringe (the diameter of the syringe is important) and about 12-13 ml blood is drawn, almost filling the syringe. If blood is required for other purposes, the use of a 21 g 'Butterfly' scalp vein set greatly eases the syringe change. Intake air in the syringe to allow mixing by gentle inversion (violent mixing diminishes platelet adhesion)[4]. The test can be performed immediately, or within an hour after remixing.

5. *Actual method* (Fig. 2). Have pump idling, and have a rack of plastic Kahn tubes ready. Tubes No. 1, 3, 5, are plain, with a 2 ml mark on them. These are discarded after use. Tubes 2, 4, 6 have 0.1 ml of 10 percent Na_2EDTA dried and powdered within, and pre-marked to 1 ml. An identical EDTA tube is also ready to receive 1 ml from unfiltered blood in syringe, for true platelet count. Release the proximal clip, select speed 4 on pump, and engage driving bar. This will give a transit time in column of 10 seconds, and a flow rate of 4.5 ml/min. Collect the blood in the tubes, and after the 9th ml has issued turn pump off. Save a ml from the syringe for a baseline PCV and platelet count. Cap and mix the 4 EDTA tubes and measure platelet counts. We obtain platelet rich plasma by sedimentation for counting on a Coulter FN.

Subtract the 3rd, 6th, 9th ml aliquot counts from true count, and calculate by

$$\frac{\text{difference}}{\text{true}} \times \frac{100}{1} = \%\ \text{adhesive platelets.}$$





6. *Normal range.* In our laboratory, the 1 S.D. range at 3, 6, 9 ml respectively are 35-80 percent, 60-90 percent, 68-95 percent. Repeated testing on one normal person showed a range of about ½ S.D. Use of a cyclostyled sheet for recording details, counts, and graphing results is convenient. The method has clearly shown decreased adhesion in von Willebrand's disease (including mild forms with normal Bleeding Times), a boy with a congenital thrombocytopathy, some myelomas, azotemia in renal failure, and ingestion of anti-platelet drugs, e.g., aspirin. Elevated adhesion can also be detected, but different speeds are currently being tried to gain better separation from normal. The purpose of this communication is to place on record for local conditions a successful adaption of the platelet adhesion method of Bowie.

I acknowledge Dr J. W. Hamer's helpful advice.

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Erythrocyte Sedimentation Rate; A Comparison of Two Methods

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Received for publication, July 1974.

Summary

A trial utilising the modified Westergren method and a plastic disposable ESR tube has shown that provided certain elementary precautions are taken, the results should be comparable with those expected from the use of the classic method as recommended by the ICSH.

Introduction

In 1973 the International Committee for Standardisation in Haematology (ICSH) published its recommendations for a reference method for Erythrocyte Sedimentation Rate (ESR)[3].

The recommended method differed from our routine method by (a) using a fluid anticoagulant and (b) using glass Westergren tubes.

In the closing stages of the ICSH report, the Committee indicate that they are not prepared to comment on (among other things) the likely effect on the ESR of 'tubes made by a material other than glass.'

For approximately 18 months, this laboratory has used a disposable polystyrene Westergren tube and cup manufactured by Guest Medical and Dental Products, supplied by Medic DDS Ltd., Wellington, and in view of the comments of the ICSH report, it was felt that any possible effects should be investigated in case they were of such magnitude as to significantly alter the ESR.

Methods and Materials

Routine Method. A Fison diluter/dispenser system incorporating an Interlink unit was adapted in such a way that it delivered four parts of EDTA anticoagulated blood plus one part of 3.13 percent (0.106 M) tri-sodium citrate ($C_6H_5Na_3O_7.2H_2O$) into the small disposable cup. A disposable Westergren tube (graduated to 150 mm) was pushed into the cup in such a way that the blood/citrate mixture was forced up the bore of the tube to the zero mark. Any adjustment on the zero mark was made by altering the amount of penetration by the tube into the cup. The unit was allowed to stand for exactly 60 minutes in the racks provided by the manufacturer. Appropriate precautions as to verticality, ambient temperature and other physical conditions were taken.

Reference Method. 4 ml of the patient's blood was collected into specially prepared stoppered polystyrene test tubes containing 1 ml of 3.13 percent tri-sodium citrate. The remainder of the test procedure followed the ICSH recommendations.

Suspended Variation of Routine Method. The racks as provided by the manufacturer do not always allow the tubes to remain exactly vertical. In view of this we suspended a small batch of specimens in parallel with the previous method to see if any significant discrepancies occurred. The method varied from our routine method only in so far as the tubes were supported at the top by inserting a $1 \times \frac{3}{32}$ inch brass cotter pin into the lumen of the tube. Each tube was then suspended from a small curtain hook attached to an overhanging shelf. The results were recorded after one hour.

Paired samples were collected into EDTA and sodium citrate from random inpatients suffering from a wide variety of diseases. A number of normals (staff) were included. To check the precision of the measurement, a large volume of blood was collected into EDTA and into sodium citrate from two patients with known elevated sedimentation rates. These samples were tested within two hours of collection.

Results

Precision. Statistical analysis of specimens tested in parallel by three different methods is shown in Tables I and II.

Method Correlation. A total of 84 random ESR's were compared by the ICSH and Routine Method. The distribution of these results is shown in Figures 1 and 2.

SLIGHTLY ELEVATED E.S.R.

METHOD	SAMPLE NUMBERS	MEAN VALUE	1 S.D.	C.V. %	95% CONFIDENCE LIMITS (MEAN ± 2 S.D.)
I C S H	18	28.4	1.84	6	24.72 TO 32.08
ROUTINE	15	28.3	1.63	5	25.04 TO 31.56
SUSPENDED	14	27.2	2.01	7	23.18 TO 31.22

Table I.

MODERATELY ELEVATED E.S.R.

METHOD	SAMPLE NUMBERS	MEAN VALUE	1 S.D.	C.V. %	95% CONFIDENCE LIMITS (MEAN ± 2 S.D.)
I C S H	18	48.4	2.89	6	42.62 TO 54.18
ROUTINE	11	53.63	3.23	6	47.17 TO 60.09
SUSPENDED	13	50.07	1.89	4	46.29 TO 53.85

Table II.

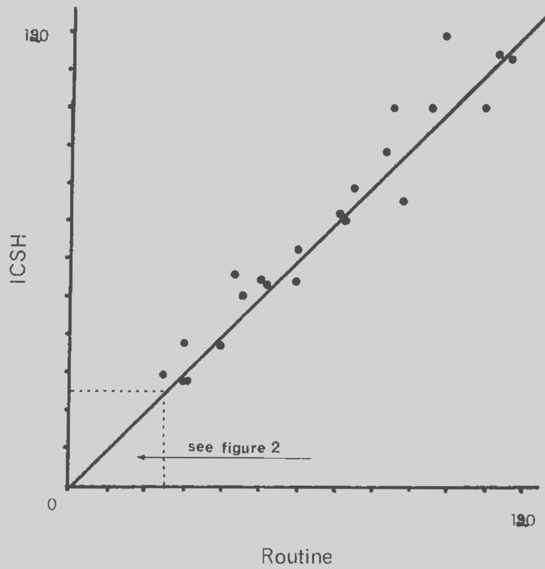

Figure 1. ICSH V Routine Method.
(greater than 25mm)

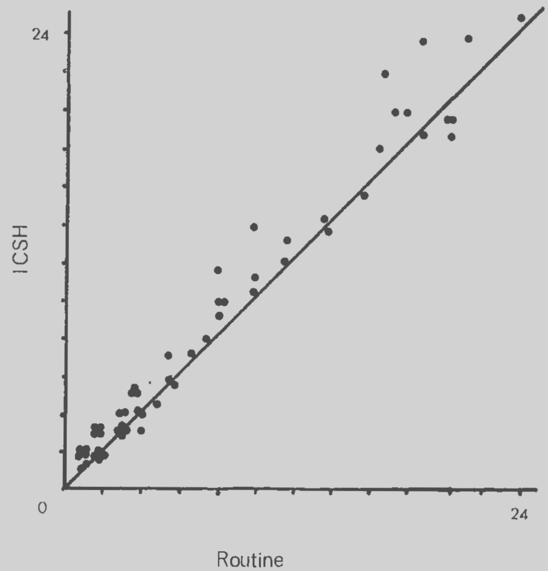

Figure 2. ICSH V Routine Method.
(less than 25mm)

From Figures 1 and 2 it can be seen that most of the results fall slightly in favour of the ICSH method. Assuming the ICSH result to be the true value statistical studies were applied to detect any significant variants which would mitigate against continuing with our present disposable system.
Statistical analysis yielded—

Correlation Co-efficient (R*) — 0.991362
Constant error — + 1 mm
Proportional error — 3.1 percent

These figures confirm that the routine results will be constantly 1 mm less than the ICSH results and that over the range of samples measured, a proportional error of 3 percent was encountered.

* Where for perfect correlation R should = 1.

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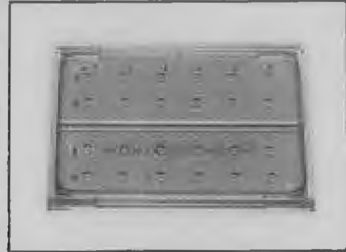

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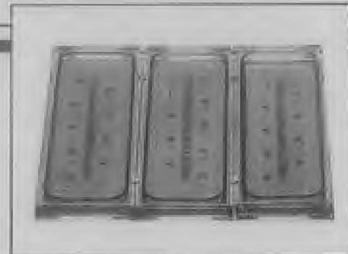

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Discussion

Since 1918 when Fahraeus discovered the accelerated rate of sedimentation of red cells in pregnancy and other disorders many methods of ESR measurement have been advocated. However, for a variety of reasons, the majority have not stood the test of time. In 1965 the ICSH established an expert panel to bring down recommendations for the standardised measurement of the ESR. The change in method by this laboratory was made in view of impending hygiene regulations. After looking at the available materials, it was felt that the disposable system offered certain clear-cut advantages despite the small increase in cost.

Briefly, these advantages were:

1. Cessation of mouth pipetting and removal of the associated hepatitis risk.
2. Removal of the risk to staff of gashed hands from the broken ends of the glass pipettes whilst putting them into the racks.
3. No further need for regular preparation and subsequent handling of Acid-Dichromate solutions.
4. Other well known advantages of using a disposable system.

In 1973 the ICSH recommendations were published and this stated that the method of choice was that of Westergren (collected into sodium citrate). In the Appendix to these recommendations, the authors said, among other things, that insufficient evidence was available as to the effect of solid anticoagulants and tubes made from materials other than glass.

In view of our method this study was undertaken.

It is now general practise to collect specimens for routine blood counts into EDTA and it would be both unreasonable and unacceptable to collect separate specimens for the determination of the ESR. Dawson[1] in his paper outlines the reasons for using EDTA as the anticoagulant of choice and Hazelton[2] is in agreement, although he adds two other chemicals which he claims will not affect this determination.

The use of various kinds of 'plastics' has, over the past 10 years, increased dramatically, yet the relationship of some of these substances to biological situations is not clearly established. In view of this growing use it is surprising that other foreign manufacturers have not produced a similar item, and it has been suggested that the reason lies in the reluctance by these firms to guarantee the correctness of the product when delivered to the consumer. For example, it has been claimed that warping of the tubes may occur in transit, thus altering the final result. This has not been borne out by our experience and I would suggest that warping of such magnitude to alter the result would be sufficiently obvious to the worker that they would not use that particular tube or batch.

The internal dimensions of the tubes were in accordance with the ICSH recommendations.

The ability to store blood overnight in EDTA is a well-documented advantage and a number of authors have shown that this storage will not adversely affect the ESR provided the bloods are well mixed and brought back to room temperature prior to testing (Melville[4], Dawson[1], Hazelton[2]).

In view of this it seems a little strange that the ICSH see fit to recommend storage at 4°C for not more than six hours. During this study, careful attention was paid to the verticality of the tubes as any deviation has been shown to significantly alter the final result.

To check to what extent this may be a problem, a short experiment was undertaken which consisted of suspending a series of tubes as described. The results are shown in Table I and show that provided adequate precautions are taken, this offers no real advantage short of some saving in actual usable bench space. Bench vibration has, in the past, been considered to be a major source of false results: however, this has been ruled out by some simple experiments by Dawson[1] and Hazelton[2]. The role of sodium citrate in the current modification of the classic Westergren method has always been somewhat in doubt: however, Dawson[1] and Hazelton[2] have shown that in fact this can be replaced with isotonic saline with no detrimental effect.

Cleanliness of the lumen of the glass tubes has been another factor well learnt by trainees as having an effect on the result. Dawson and others show that provided these tubes are thoroughly washed in water and dried then this is not a problem. This situation does not, however, arise in this disposable system.

Acknowledgments

I am indebted to Miss R. Ball for her technical help, to Mr T. Wilson and Mr G. Cathro for their statistical studies and Mr A. Nixon for his continuous assistance and encouragement.

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The National Health Institute Culture Collection

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National Health Institute, Wellington

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The National Health Institute, Wellington, is one of a number of laboratories in New Zealand which maintain extensive culture collections of micro-organisms. The present NHI collection consists of approximately 1,000 named species, biotypes and serotypes, the majority of which are bacteria of medical significance. A limited number of bacteria of veterinary importance are included particularly of they have significance as a cause of zoonotic infection.

Many of the cultures are derived either from the National Collection of Type Cultures (NCTC) in England or the American Type Culture Collection (ATCC) in the United States of America. Catalogues of both these collections[1, 2] are held at NHI together with a recently published World Directory of Collections of Micro-organisms[3]. Some recent isolates of clinical significance from laboratories such as the Centre for Disease Control, Atlanta, Georgia, USA, are also available.

A New Zealand Reference Culture Collection of Micro-organisms is being established by the Department of Scientific and Industrial Research, on the advice of the New Zealand Committee on Culture Collections of Micro-organisms[4]. Cultures for deposit will include well authenticated micro-organisms of nomenclatural type or neotype of organisms described in the literature. The National Health Institute has been designated as the depository for organisms pathogenic to man or of human origin.

A directory of Collections and Lists of species maintained in New Zealand has been published as part of the Commonwealth Collections of Micro-organisms[5] but this is now somewhat dated. The NHI is preparing a catalogue of cultures available for distribution and it is envisaged that an annual list of additions will be circulated to laboratories and other interested persons. No charge is made for cultures unless they are to be used for commercial purposes. There are few restrictions on the release of cultures from the collection, providing the curator is satisfied with the competence of the person(s) requesting them. Some cultures such as *Brucella melintensis,* however, would not be released as this organism is apparently not present in New Zealand. The direct importation of micro-organisms into New Zealand requires a prior permit from the Animal Health Division of the Ministry of Agriculture and Fisheries, Wellington. Supporting data are required for each application. Requests are occasionally made to the Ministry to import cultures when the NHI or some other repository in the country may well have the culture available. It is suggested that initial enquires be directed to the NHI so that if a culture cannot be supplied locally the curator may be able to assist in obtaining them elsewhere.

Requests for cultures should be made to the Curator (presently Dr R. A. Robinson), NHI, and should specify the micro-organisms required using preferably genera and species according to Bergey's Manual of Determinative Bacteriology (7th Edition).*

For more recently characterised species, synonyms should be included. The purposes for which cultures are required should also be stated, e.g., teaching, research, or commercial uses. If an organism requested is for a specific test or procedure, this should also be stated in the application, as alternative micro-organisms may be available where the requested strain is not.

Freeze-dried cultures are supplied with specific directions for opening ampoules. No culture should be assumed to be non-pathogenic, and appropriate precautions should be taken at

* The 8th Edition of this Manual is scheduled for publication in late 1974.

all times. The curator would appreciate being notified if cultures received are contaminated, non-viable, or not authentic. Occasionally difficulties are experienced in growing and maintaining freeze-dried cultures. If the organisms are not considered fastidious then a few drops of nutrient broth added to each ampoule containing the filter paper strip should be adequate. This is then plated on to a suitable solid medium and the remaining broth (and paper strip) incubated at the appropriate temperature. Incubation for periods of longer than 24 hours may be required for some micro-organisms. Heart infusion broth may be preferable to nutrient broth. Bacteria such as *Haemophilus* sp. or *Neisseria* sp. are better revived with 7.5 percent glucose serum and plated directly on to chocolate agar.

For the maintenance of stock strains we recommend the following media at room temperature in the dark (such as a drawer):—

Cooked Meat Broth:—
- Bacillaceae (especially *Clostridia* sp.)
- Lactobacillaceae (especially Streptococcus pyogenes, and Peptostreptococcus)
- Bacteroidaceae
- Actinomycetaceae

Dorset's Egg Slope:—
- Enterobacteriaceae
- Corynebacteriaceae
- Mycobacteriaceae

Nutrient Agar Slopes:—
- Enterobacteriaceae
- Pseudomonadaceae
- Micrococcaceae
- Spirillaceae (*Vibria* sp.)

Blood Agar Slopes:—
- Brucellaceae
- Lactobacillaceae

Chocolate Blood Agar Slopes:—
(subculture frequently)
- Brucellaceae (*Haemophilus* sp.)

Dextrose Starch Agar Slopes:—
(stab with Ondina Oil overlay kept at 37°C.)
- Neissariaceae

Fletcher's Medium:—
- Treponemataceae (especially *Leptospira* sp.)

For further information on the maintenance of bacteria the paper of Lapage *et al.*[6] contains useful information. Stock or reference cultures should not be subcultured continuously; the original subculture made from the freeze-dried ampoule should be used whenever possible.

Occasionally individual laboratories refer cultures directly to overseas reference laboratories for identification, confirmation, or further subdivision on the basis of serotype, phage, or colicine typing. Where convenient, it is desirous that this be done through the National Health Institute to ensure that cultures of micro-organisms of local importance are preserved, and the type culture collection is representative. Where for any reason this is not suitable, duplicate cultures and results should be made available to the culture collection, so that the clinical significance of less common organisms of medical importance are recorded for the future use of other laboratory workers encountering similar problems.

Acknowledgment

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A Rapid Microbiological Assay for Gentamicin

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Received for publication, August 1974

Summary

Aminoglycosides can be assayed in 2½ to 4 hours using an agar diffusion method. Assay plates could be stored for up to 96 hours before use. Any penicillin or cephalosporin mixed with aminoglycoside could be neutralised by a new commercially available β-lactamase.

Introduction

In the last few years aminoglycosides have become widely used in the treatment of serious infections. As a result the assay of aminoglycosides has become necessary because of their potentially toxic properties.

The ideal assay method for aminoglycosides should be simple, so that it can be used by all laboratory personnel, and rapid so that accurate regulation of therapy is possible. It should also allow the measurement of aminoglycoside in the presence of penicillins or cephalosporins as these antibiotics are often administered in conjunction with aminoglycosides.

For the last year the Microbiology Department at Green Lane Hospital has used, without problems, an assay method that meets these requirements. The method was adapted from that of Grove *et al.* (1955)[2] for the assay of streptomycin. It was simplified by storage of assay plates and incubation time was shortened from 18 h to 2½-4 h (usually 3 h). The assay of aminoglycoside in the presence of penicillins or cephalosporins was made possible by using a commercially available, stable, β-lactamase preparation described by Newsom *et al.* (1973)[3] and Waterworth (1973)[5].

The method was also found to be suitable for the assay of penicillins when rapid estimations of blood levels were required for the regulation of therapy.

Materials

Spores of *Bacillus subtilis* ATCC 6633 were prepared essentially as described by Grove *et al.* (1955)[2]. Stock suspensions contained 10^{10} spores per ml and could be stored at 4°C for at least 6 months. Antibiotic medium 5 (Grove *et al.*, 1955)[2] was used for the assay of aminoglycosides, and seed agar antibiotic medium 1 (BBL) for the assay of penicillins. Disposable, sterile, plastic petri dishes 9 cm in diameter were used as assay plates. Stock solutions of antibiotics were prepared in phosphate buffer pH 7.0 for penicillins and pH 7.9 for aminoglycosides. Working standards for the assay of serum were made up from these using plasma or serum. A zinc stabilised β-lactamase preparation derived from *Bacillus cereus* 569/H cultures (Sabath *et al.*, 1971)[4] was used to inactivate penicillins and cephalosporins. Commercial preparations were obtained from Whatman Biochemicals. A number 5 cork borer (9 mm in diameter) was used to cut wells in the assay agar.

Methods

A 1 ml volume of *B. subtilis* spore suspension was added to 200 ml of assay agar which had been cooled to a temperature between 45° and 50°C. Aliquots of 25 ml were poured into petri dishes on a level surface giving a depth of 4 mm.

Immediately the agar had solidified the plates were stored upright, in sealed plastic bags, at 4-8°C to prevent water loss and premature germination of the assay micro-organism.

Assays were run in triplicate with 5 antibiotic standards plus 1 or 2 unknowns to each plate.

Sera that contained aminoglycoside mixed with penicillins or cephalosporins were neutralised with β-lactamase used according to the maker's instructions.

Wells were cut in the assay agar and filled with equal volumes of standard and unknown administered dropwise from a Pasteur pipette.

The plates were then left to diffuse at room temperature for no longer than 30 minutes (Ericsson *et al.*, 1971)[1] after which they were incubated at 37°C until zones of inhibition were clearly visible (2½-4 h, usually 3 h).

Zones of inhibition were measured using calipers or an extended 'Partigen' immunoelectrophoresis zone reader. The mean of the diameter for each antibiotic standard was plotted against concentration on semi-log graph paper, the concentration on the Y axis and the mean zone diameter on the X axis. The points on the graph obtained from the standards formed a straight line from which the concen-

Table I
The Effect of Storing Antibiotic Assay Plates Between 4 and 8°C for 24, 48, 72 and 96 hrs.

Concentration of Gentamicin µg/ml	0 h at 4-8°C Test	24 h at 4-8°C Test	24 h at 4-8°C Control	48 h at 4-8°C Test	48 h at 4-8°C Control	72 h at 4-8°C Test	72 h at 4-8°C Control	96 h at 4-8°C Test	96 h at 4-8°C Control
	Mean Zone Size in mm after:—								
0.5	19.1	19.1	19.1	19.0	19.1	19.1	19.1	19.0	19.0
1.0	19.8	19.9	19.9	19.8	19.9	19.9	19.9	19.8	19.9
2.0	20.8	20.9	21.0	20.8	20.7	20.7	20.7	20.7	20.7
4.0	21.6	21.6	21.7	21.5	21.7	21.6	21.7	21.6	21.6
8.0	22.3	22.2	22.3	22.3	22.3	22.2	22.3	22.3	22.3

tration of the unknown could be read.

The effect of the storage of assay plates between 4 and 8°C was assessed by comparing the zone diameters of a series of antibiotic standards assayed on stored plates with the zone diameters of the same antibiotic standards assayed on freshly poured control plates at intervals of 24, 48, 72 and 96 h.

Results

The mean zone sizes of five gentamicin standards measured on test plates after storage at 4-8°C for varying lengths of time are shown in Table I. The mean zone sizes of the five gentamicin standards measured on the corresponding control plates are also shown.

Discussion

There was no appreciable variation between the mean zone size of gentamicin standards on control plates and test plates. Thus it is possible to store antibiotic assay plates at 4-8°C for periods of up to 96 h and still produce results as reliable as those from freshly poured plates.

This means that assays of aminoglycosides and penicillins can be carried out in busy laboratories immediately blood for assay arrives and results can be obtained in 2½-4 h. In this way accurate regulation of therapy of patients with renal failure being treated with aminoglycoside antibiotics is possible and toxic side effects of treatment may be prevented.

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Hepatitis-Associated Antigen Contamination in Syphilis Serology Control Sera

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Received for publication, April 1974

Summary

Care should be taken in syphilis serology laboratories when selecting commercially available positive control sera of human origin as hepatitis-associated antigen has been demonstrated in six of seven products from five manufacturers using a sensitive radioimmunoassay technique.

Introduction

The growing awareness of the frequency of hepatitis-associated antigen (HAA) contamination in certain commercially obtainable laboratory reagents is largely due to the recent availability of a simple and sensitive radioimmunoassay (RIA) procedure. Clinical chemistry control and reference preparations, probably by virtue of their quantity and variety, have been especially implicated[1, 2, 5, 6]. Less notable has been the contamination of positive control sera for syphilis serology testing. In a broader and more diverse survey recently completed[2] it was noted that several such reagents contained HAA. It was decided to extend this facet of the investigation in order to further evaluate the existence of HAA in this area of quality control.

Materials and Methods

Several control preparations were purchased from five manufacturers of which four were USA-based and one Italian in origin. Lyophilised reagents were rehydrated as instructed and tested using a solid phase radioimmunoassay procedure marketed by Abbott Laboratories (Ausria-125). This method as originally described involved overnight incubation of test

Table I

Details and results of seven syphilis serology control sera tested for HAA by radioimmunoassay, complement fixation and immuno-electro-osmophoresis.

Sample Number	Manufacturer	Lot Number	Radioimmunoassay CPM	Cutoff value	Antigenicity	Result	CF	IEOP
1	Dade	PS-82CB	3373	481	7.0	+	AC	0
2	Dade	PS-82CD	3047	191	16.0	+	0	0
3	BBL	0091910	1104	479	2.3	+	0	0
4	BBL	1101601	2408	355	6.8	+	0	0
5	Hyland	0360T002A1	294	246	1.2	+	0	0
6	Sylvana	010273-1B	151	233	<1.0	0	0	0
7	Italdiagnostics	2530	2318	326	7.1	+	AC	0

Key— 0: Negative. +: Positive. AC: Anticomplementary. CPM: Counts per minute.

Antigenicity: Used here to express the antigen content of RIA-Positive sera relative to their CPM and may be calculated by dividing the CPM values of reactive sera by the cutoff value.

samples at room temperature. Samples 1, 3 and 4 were assayed in this fashion. Latterly a modified methodology was advocated by the manufacturers which entailed a substantially shorter incubation period at 45°C and was used to examine samples 2, 5, 6 and 7. Control sera positive by the room temperature RIA procedure were subjected to neutralisation testing using human anti-HAA. No attempt was made to inhibit those control samples reactive by RIA at 45°C. A full description of both the Ausria-125 RIA and RIA-inhibition techniques appears elsewhere[2]. All control preparations positive for HAA by either RIA method were further examined by complement fixation (CF)[2] and immuno-electro-osmophoresis (IEOP) (Austigen II, Hyland Laboratories).

Results and Discussion

As will be seen from Table I six of seven control reagents tested were positive by RIA (85.7 percent) while HAA was not detectable in any sample by either CF or IEOP. One serum positive by RIA was only marginally reactive as evidenced by the low antigenicity. The antigenicities of reactive samples varied from 1.2 to 16.0.

The specificity of Ausria-125 as originally described by Abbott Laboratories has been challenged by several investigators[3, 4]. Accordingly, samples positive by this earlier procedure (i.e., 1, 3 and 4) were subjected to neutralisation testing following the recommendations of Prince *et al.* (1973). All showed immunologically specific inhibition following pre-incubation with anti-HAA of human origin. Those remaining samples (i.e., 2, 5, 6 and 7) were assayed by means of the modified RIA procedure referenced previously which, it is claimed by the manufacturers, lessens the possibility of false positive reactions. No inhibition testing was undertaken in this latter group.

Attention is thus drawn to what appears to be a common incidence of HAA in commercially acquired syphilis serology control sera. It has been assumed that all preparations are of human origin and represent a serum pool although this was not always stated. In no instance was the date of manufacture recorded in accompanying data and neither was any reference made to specific testing for HAA by any recognised procedure. Until otherwise established it would seem prudent that evidence of HAA in such reagents should be taken as an indication of potential infectivity.

Acknowledgments

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Addendum: Since this paper was forwarded for publication two further syphilis control reagents have been tested. One (Boeringer Lues Control Serum, Lot 37D) was negative by radioimmunoassay while the other (Lee Laboratories Syphilis Control Serum, Lot 10-1214) was positive for hepatitis-associated antigen by the same procedure. The latter product possessed an antigenicity of 12.5.

Technical Communications

Urine Protein Screening

Over a period of ten days, 24h urines for total protein estimation were tested with Albustix and a quantitative urine protein method. The results obtained are presented.

The method used to routinely quantitate urine proteins at the Department of Clinical Biochemistry, Christchurch Hospital, is that of Savory, Pu and Sanderman[1]. Although it is an extremely accurate method for urine protein quantitation, it is also a tedious method for large numbers of urines. It was decided to examine the validity of screening the urines with Albustix (Miles Laboratories, Australia) before proceeding to quantitate the urine proteins in an attempt to reduce the number of urines for quantitation.

A total of 145 urines were tested. The urines were acidified with concentrated hydrochloric acid to approximately pH6.0 and tested with Albustix, prior to quantitation. The urines were tested with Albustix in accordance with the directions given on the product container and compared with the comparitor on the container. Any quantitative protein results below 10 mg/dl were classed as negatives.

The results obtained were as follows:

ALBUSTIX FALSE POSITIVES

1. Results which gave 30 mg/dl with Albustix but were less than 30 mg/dl when quantitated. 2
2. Results which gave a Trace of protein with Albustix but were less than 10 mg/dl when quantitated 11

ALBUSTIX FALSE LOW VALUES

1. Results which gave a Trace of protein with Albustix but were between 30-100 mg/dl when quantitated 13
2. Results which gave a Trace of protein with Albustix but were over 100 mg/dl when quantitated 1
3. Results which gave 30 mg/dl with Albustix but were over 100 mg/dl when quantitated 8
4. Results which gave 100 mg/dl with Albustix but were over 300 mg/dl when quantitated 1
5. Results which gave 100 mg/dl with Albustix but were over 1,000 mg/dl when quantitated 1

ALBUSTIX FALSE NEGATIVES

1. Results which were negative with Albustix but were positive when quantitated 44

In all 81 false results were found out of 145 urines, this is summarised below:

		%
False positives	13	9.0
False low values	24	16.6
False negatives	44	30.3
False high values	nil	——

A comparison of quantitative urine protein methods is currently being undertaken and will be submitted for publication shortly.

M. LEGGE,
D. DOHRMAN,
Dept. of Clinical Biochemistry,
Christchurch Hospital.

August, 1974.

REFERENCE

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Polymerised Silicone Oil in Autoanalyser I Heating Baths

The use of continuous flow analysis for the determination of estriol in pregnancy, Lever *et al.* (1973)[1], involves reaction temperatures greater than 100°C. Because Autoanalyser I heating baths are not sealed units, the use of either transformer or high temperature mineral oils is unacceptable since they produce unpleasant fumes. Most workers now use silicone oil (dimethyl polysiloxanes), which, although expensive at approximately $25 per 4.5 Kg, have special properties of inertness and non-toxicity.

Nevertheless, our laboratory has experienced an occasional problem with polymerisation of silicone oil. Contamination with sulphuric acid from the Kober re-agent is suspected.

The most effective and reasonably-rapid technique of cleaning was found to be:

(a) Physical removal of polymerised oil.

(b) Stripping contaminated parts into basic components and then:

(1) Cleaning pot assembly and stainless steel parts using steam, or alternatively, "Ajax" cleanser or an equivalent agent to absorb polymer. This forms a sludge which is then easily removed.

(2) The glass coil is effectively cleaned by soaking in chloroform, ether, or dichloromethane.

J. POWELL.
Green Lane Hospital.
Auckland.

May, 1974.

REFERENCE

1. LEVER, M., POWELL, J. C. and PEACE, S. M. (1973). *Biochem. Med.* 8, 188.

Transferred Drug Resistance

In July, 1974, a 19-year-old girl with food poisoning had *Salmonella newington* isolated from her faeces. Sensitivity to antibiotics was performed on the salmonella by the Stokes Method with a control-sensitive organism. The *Salmonella newington* was shown to be sensitive to ampicillin, among other antibiotics, and the patient was given one week's course of ampicillin. Nine days from the first isolation, *Salmonella newington* was again isolated from her faeces and repeat sensitivities showed complete resistance to ampicillin and to tetracycline. Two further isolations showed, in addition, partial and then complete resistance to ceporan.

At the time of the fourth isolation of *Salmonella newington* various non-salmonella organisms from the patient's faeces were tested for sensitivity to various antibiotics. This revealed the presence of an *E. coli* which was resistant to ampicillin, tetracycline and ceporan: sensitive to cotrimoxazole and kanamycin (See Table 1).

This rapid change in sensitivities to three antibiotics took place after a course of ampicillin for one week. No other antibiotics were given to the patient during this period or for many years prior to the salmonella infection. The results strongly suggest that multiple drug resistance was transferred from the *E. coli* in the patient's bowel to the invading *Salmonella newington.*

B. M. LOCKWOOD,
Palmerston North Hospital.

August, 1974.

Table 1

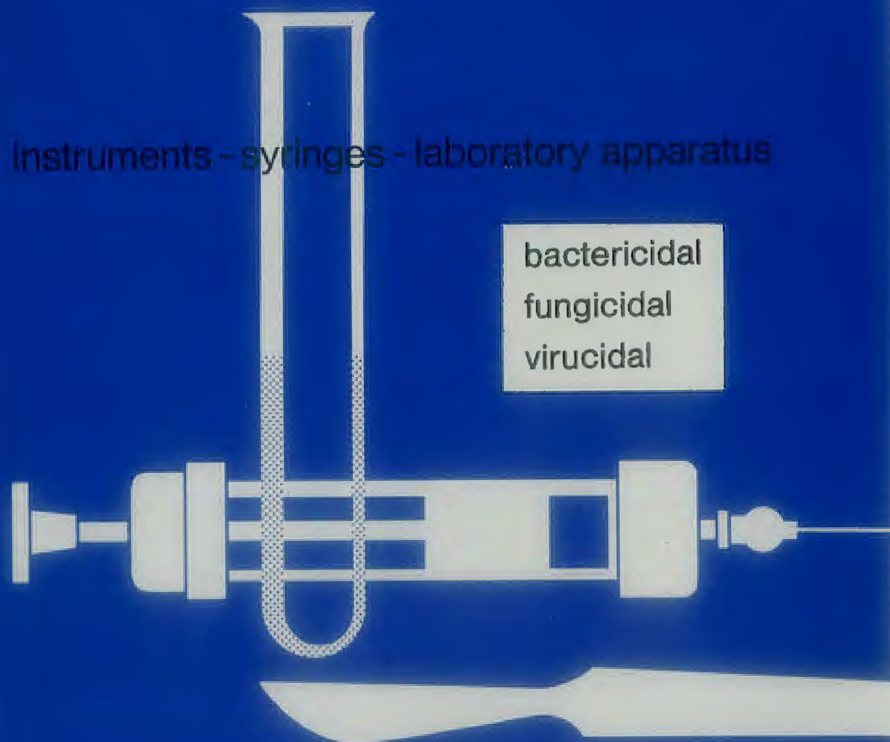
Date	Culture	Ampicillin	Tetracyline	Ceporan	Cotrimoxazole	Kanamycin
8.7.74	Salmonella newington	++	++	++	++	++
17.7.74	Salmonella newington	–	–	++	++	++
23.7.74	Salmonella newington	–	–	+	++	++
24.7.74	Salmonella newington	–	–	–	++	++
24.7.74	E. coli	–	–	–	++	++

It is well-known that some shigellae and salmonellae have, while in the intestines of carriers, acquired multiple drug resistance by infection with a 'resistance transfer factor' derived from commensal E. coli. This appears to be one of those cases. It would have been interesting to see if the original sensitive Salmonella newington strain became resistant when cultured alongside the resistant E. coli.—M.J. sub-editor.

Hepatitis B Antigen Methodology

Although counterelectrophoresis (CEP) is probably the most commonly employed hepatitis B antigen (HBAg) detection system in this country it is a method of only moderate sensitivity. The introduction of more sensitive procedures offer attractive alternatives. Since July 1973 all sera forwarded to this laboratory for HBAg screening have, in the main, been assayed by radioimmunoassay (RIA) in parallel with CEP. The RIA method used is that of Abbott Laboratories, initially as Ausria-125 and latterly its modification, Ausria II-125. Austigen II, marketed by Hyland Laboratories, comprised the CEP system. During the ensuing period 67 HBAg-positive individuals have been detected by RIA. Of these, 44 (66.6 percent) were also positive by CEP. Fifty-one of the 67 RIA-positive patients were found using Ausria-125 and 38 (74.5 percent) of these were reactive by CEP. The remaining 16 were detected by the modified and more sensitive Ausria II-125. Only six (37.5 percent) of this group however were positive in the electrophoresis procedure. In no instance was a CEP-positive serum unconfirmed by RIA.

It is apparent that a considerable proportion of those with hepatitis B antigenaemia will remain undetected if CEP-type methods only are used. There is good evidence[3] that donated blood that is negative for HBAg by CEP but



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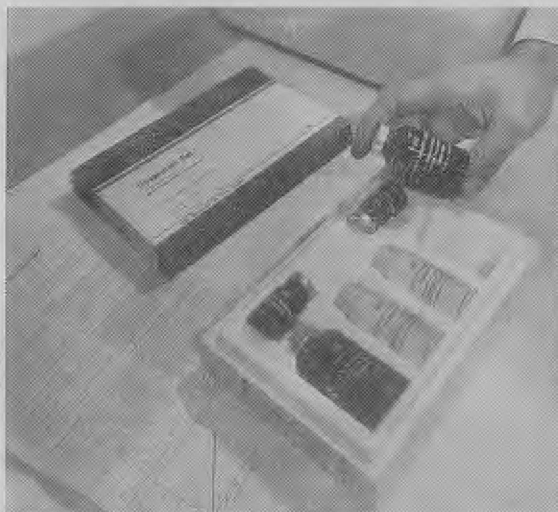

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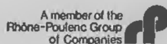

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positive for this virus by RIA is capable of transmitting one form of post-transfusion hepatitis. The requirement for sophisticated counting equipment for a moderately expensive testing system may discourage the establishment of RIA in some laboratories, especially for mass screening. However the more recent development of a direct haemagglutination test using turkey erythrocytes coated with purified anti-HBAg[1] may offer a satisfactory alternative. It is claimed[1, 2] that while only slightly less sensitive than RIA this method is rapid, simple and economical. It is applicable to mass screening and is considerably more sensitive than CEP. A procedure of this nature may well become the method of choice for both large and small-scale HBAg monitoring.

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M. J. GRATTEN,
Department of Immunology,
SHERYL YOUNG,
Department of Microbiology,
Pathology Services,
Christchurch Hospital.

September 1974.

The Histology Cryostat—a Low Temperature Incubator?

Personnel who work in histology laboratories are all aware of the merits of the particular model of cryostat they have and the care they take, following the manufacturer's directions, to ensure they remain in functional order.

However, not much attention has been paid to the periodic disinfection of the cryostat, either from the manufacturers themselves or from current histology text-books.

This then prompted questions regarding the tissue trimmings from frozen sections lying within the cryostat.

1. Could some of these trimmings possibly contain pathogenic bacterial when cut from infected tissue?
2. Would these organisms be able to remain viable at the low cryostat temperature of —20°C until the cryostat was next cleaned and defrosted?

If so, they posed a potentional health hazard to laboratory personnel. The answer to these questions forms the basis of this technical communication.

The viability of two common micro-organisms to withstand the low cryostat temperature in a broth culture was first determined.

Pure colonies of *Staphylococcs aureus* and *Escherichia coli* were inoculated into trypticase soy broth and placed within the cryostat interior. The frozen cultures were then thawed, sub-cultured on to recovery medium and the cultures frozen again for a period of three weeks.

Growth resulted from each daily sub-culture proving that these two organisms could remain viable at —20°C in an enriched medium.

A piece of sterile kidney measuring 1.5 × 1 × 0.5 cm was now inoculated with drops of bacterial suspension from each organism and placed within a sealed container inside the cryostat. This was to simulate a piece of tissue requiring urgent frozen section. This frozen tissue was then cut daily on the cryostat for a period of three weeks, the trimmings being collected asceptically into trypticase soy broth.

Overnight incubation resulted in subsequent re-growth of the organisms, demonstrating that these organisms did remain viable at the low cryostat temperature when present in tissue.

From these observations it appeared there was a need for periodic disinfection of the cryostat interior and for aseptic precautions to be practised.

Various disinfectants were applied to the cryostat interior after defrosting, e.g., Medol, Lysol, Alcohol and Savlon. Absolute alcohol was favoured as it was found to be the most thorough in killing the organisms in the broth cultures, was easy to use and was readily available in the laboratory.

An ideal method, however, would be the incorporation of an ultra-violet light into the cryostat at the time of manufacture. This would be switched on for the pre-determined time while the cryostat was defrosting; thus eliminating the dangers due to the possible presence of micro-organisms in tissue within the cryostat.

K. J. ALDERTON,
Pathology Department,
Christchurch Hospital.

May, 1974.

Book Reviews

The Revolution in Medical Technology Education. Robienetta Driver, MA, MT(ASCP), and Mary A. Feeley, MS, MT(ASCP), 1974. Published by Charles C. Thomas, Springfield, Illinois. Price, $US7.95. 59 pages.

The revolution referred to in this little book is the fundamental change in attitude discerned in the American scene to the education of the medical technologist. The development and ever-increasing complexity of medical laboratory work has been paralleled by the proliferation of grades of staff and areas of certification.

In the States there are three general levels of certification under the auspices of the American Society of Clinical Pathologists. (There are other recognised methods of qualification and recently a National Accrediting Agency for Clinical Laboratory Science has been set up.) The three levels are Certified Laboratory Assistant granted after one year of post high school approved laboratory training; Medical Laboratory Technician, an associate of arts degree or its equivalent with one year of laboratory training and the Medical Technologist who undertakes a Baccalaureate degree programme of four years' duration. Progress through the various levels is possible.

The transition which is taking place is from training to education. The ability to recall and describe a number of routine techniques scarcely fits the technologist for the work he is required to undertake now, nor does is provide him with the knowledge and skill to adapt himself to the changing needs of the future. The role of the medical technologist is fluid and is influenced by the type and size of the laboratory. He may be involved in administration, laboratory management, laboratory planning, teaching and interpretation. He is thrust into many difficult situations because there is no one else there to cope with them. Unfortunately there may be rather tardy recognition of these facts and insufficient enthusiasm for the type of educational programme required to provide people of the requisite calibre. In America it appears that the four year format has been adopted as the best method of producing the type of person required. This seems to consist of three years study in an accredited institution which may be a college, university or hospital followed by a final clinical year. Minimum requirements are chemistry including organic or biochemistry, biology including microbiology, immunology, physiology, genetics and anatomy. Also mathematics, physics and statistics. This is usually followed by the major subject area of medical technology. This book describes the developments in the field of education from the setting up of the Board of Registry of Medical Technologists by the American Society of Clinical Pathologists in 1928 and the complementary Board of Schools. The roles of the various personnel involved in teaching are explained in some detail and the difficulties and obstacles which have to be dealt with while fulfilling these roles. Both the Programme Director and the Educational Co-ordinator must be well qualified and have considerable teaching experience. The authors of this book are both Educational Co-ordinators and in fact educational terminology is much in evidence. This sometimes is difficult to follow. To quote, 'Education is the process of changing behavioural patterns and curriculum is the mechanism for effecting this change. According to educational professionals curriculum consists of the method used to achieve behavioural objectives. Many find these words difficult to accept . . .' I feel tempted to add, or understand! I think it means that the pupil should finish up with the correct attitudes and motivation. There are brief chapters on desirable qualities for a teacher, methods of teaching and teaching aids, student selection and the need for continuing education.

This book is essentially a justification for education in depth for medical technologists with a description of the facilities and staff required and a discussion of the problems. It should prove of interest to those involved in education.

R.D.A.

Manual of Clinical Laboratory Methods, fourth edition, eighteenth printing, 1973. 416 pages, 675 illustrations, 8 in colour. $US14.75. Published by Charles C. Thomas, Springfield, Illinois, USA.

Judging by the number of times this book

has been reprinted it would appear that it is still in vogue in the States and indeed the author is held in high regard. However, while some parts of it are still useful a textbook written in 1949 must of necessity be woefully out of date. The clinical chemistry predates not only automation but flame photometry and quality control receives no mention here.

The blood group serology recommendations are by present standards inadequate if not outright dangerous. The haematology section contains some useful information but there is no mention of the universally accepted cyanhaemoglobin technique and the Westergren ESR technique contravenes many of the ICSH recommendations. The colour plates on malaria are good but the plates showing maturation of blood cells bear no resemblance to the staining produced by any Romanowsky technique that the reviewer is familiar with. The microbiology section is perhaps the one that is still largely applicable and the basic clinical approach is excellent. The parasite life cycles are well set out and the cross references between specimens and techniques are helpful. While this book is not without interest the reviewers would not wish it to fall into the hands of trainees!

M.C.M., D.S.F., B.W.M., A.G.W., R.D.A.

Medical Microbiology, Volume 1, twelfth edition, Cruickshank. Copy supplied by Penguin Books (NZ) Ltd. Price, $NZ10.25.

The twelfth edition of Medical Microbiology by Cruickshank *et al.* has been awaited with considerable interest. Not only is it the first edition of this popular textbook since 1965, but it also introduces a new concept. As explained in the preface, the expanded text in the eleventh edition had given the book a 'middle-aged spread' and the large amount of space devoted to laboratory procedures made it unattractive to medical and science students and doctors. For these reasons it was decided to publish Medical Microbiology in two volumes; the first volume aimed primarily at students and doctors, and the second volume for the professional microbiologist and technical laboratory staff.

Volume One is divided into five parts and basically covers the same ground as the eleventh edition minus most of the technical detail.

Part One deals with the anatomy of the bacterial cell, growth and nutrition of bacteria, sterilisation and disinfection, antimicrobial agents, bacterial genetics, immunology, viral structure classification and genetics.

As with most of the text in the new edition, the material in this section has been expanded, updated and is well illustrated. There are several minor disappointments. Bacterial nutrition is still treated rather superficially, the section on sterilisation is essentially the same as in the previous edition, while the text on disinfectants, although enlarged, could have been dealt with in greater depth. However, regardless of these criticisms, this is a valuable section, particularly for the student.

Part Two is devoted to bacterial pathogens and associated diseases. It deals principally with the more common bacteria, in each case giving a brief description of morphology, cultural characteristics and differentiation of each species followed by a more detailed account of laboratory diagnosis, chemotherapy, epidemology and control measures. The more common bacteria are dealt with in considerable detail, while the less common receive very brief treatment. There is much to applaud in this section but it is disappointing to find Salmonella, Shigella, E. coli, Klebsiella, Proteus and Providencia and only members of the Enterobacteriaceae group rating a mention. There is an increasing awareness of the clinical importance of other members of this group and some guidance relating to these organisms would have been in order. The description of the Pseudomonas Genus comprising more than 140 species, only one of which is pathogenic to man, would also have its critics.

I found this section rather conservative and I expected more from the 1973 edition.

Part Three relates to the more common pathogenic viruses and associated diseases. A description is given of each type of virus followed by details relating to pathogenesis, laboratory diagnosis and epidemiology. The text has been enlarged, updated and is well illustrated.

Part Four covers what may be termed 'the rest' and includes chlamydia, rickettsia, mycoplasma, pathogenic fungi and protozoa. The format is similar to the previous section. Forty-eight pages are devoted to these organisms and in some cases the descriptions are brief. There is however a surprising amount of new material condensed into this comparatively small section.

Part Five deals with the diagnosis, treatment and control of infection. An account is given of some common pathogenic conditions, their related pathogens, the nature and collection of specimens and subsequent handling in the laboratory. This is followed by the strategy of antimicrobial therapy. Bacteriologists would be pleased with the observation that samples should be collected prior to the commencement of antibiotic therapy. In the event of urgent chemotherapy being necessary, a list of bacteria possessing more or less predictable antibiograms, complete with their usual antibiotic sensitivity patterns are given. The section concludes with chapters relating to epidemiology and the control of community infections and prophylactic immunisation.

The decision to divide Medical Microbiology into two volumes certainly as related to Volume One has been a wise move.

This is a very useful textbook which has become more sophisticated while retaining the lucid style characteristic of previous editions. Both text and format make it an ideal book for the student and a most acceptable addition to the microbiologist's library.

It is true than on occasions one will have to look further for information. The authors recognise this and supply a list of references for further reading at the end of each chapter.

One waits with interest for the appearance in this country of Volume Two.

A.F.H.

Current Techniques for Antibiotic Susceptibility Testing. Edited by Albert Balows, PhD. 1974. 173 pages. Charles C. Thomas, Springfield, Illinois, USA. Price, $US13.75.

This book is one of the American Lecture Series and comprises the Proceedings of a Seminar Sponsored by Canalco Inc in 1972. The sixteen contributors present a great deal of informative data that is not only useful but an urge towards standardised procedures for performing *in vitro* antimicrobial susceptibility testing.

In the first chapter Balows emphasies the need to keep the 'Triad of Infection' in view—the host, the bacterium and the antimicrobic agent. This triangular interdependence has placed greater responsibility on the laboratory than ever before.

The agar diffusion method and particularly the Kirby-Bauer procedure is dealt with by Clyde Thornsberry in detail. Standardisation, quality control and limitation of the method.

A. L. Barry in Chapter III describes an agar overlay technique which overcomes the inconsistencies inherent in swab broth seeding.

In Chapters IV and V Wright and Barry describe efforts of the Food and Drug Administration World Health Organisation and the National Committee for Clinical Laboratory Standards to survey, review and recommend guide lines to help control the use of the disc diffusion techniques in the clinical laboratory.

John A. Washington in Chapter VI illustrates the agar dilution technique used at Mayo Clinic since the 1940's and shows the use of Steers' inocula-replicating apparatus to simplify the procedure. Again the daily use of control organisms is mentioned.

Tilton and Newberg in Chapter VIII in the Standardisation of Microdilution susceptibility testing compare methodologies, apparatus media, and environmental conditions, all of which influence the final results.

An automated microdilution method in the hand of Gavant Butler, Chapter IX, compares more than favourably with the conventional manual techniques.

The significance of quantitative testing is supported by clinical reasoning, M.I.C. tables and figures by Thrupp in Chapter X.

Vera Sutter has bravely presented methods and data that would assist most microbiology laboratories with the problem of the testing of anaerobes for antibiotic susceptibility.

Automation has been slow to come to the microbiology laboratory and in particular to sensitivity testing. The light scattering photometer by Science Spectrum Inc, the Zone Analyser by Millipore and Technicons' TAAS System are described by Thornberry and Balows and comparisons are drawn to manual methods. Imagine being able to talk about processing forty specimens per hour, with a through-put rate of three hours per specimen!

The authors' combined efforts in the 20-page appendix give an excellent run down on performance standards and evaluation recommendations.

This book is well documented, referenced and indexed and is a serious attempt not only to encourage but stress the need for a more scientific approach to a subject that for too long has been taken for granted as being fraught with biological variations difficult to control.

G.D.C.M.

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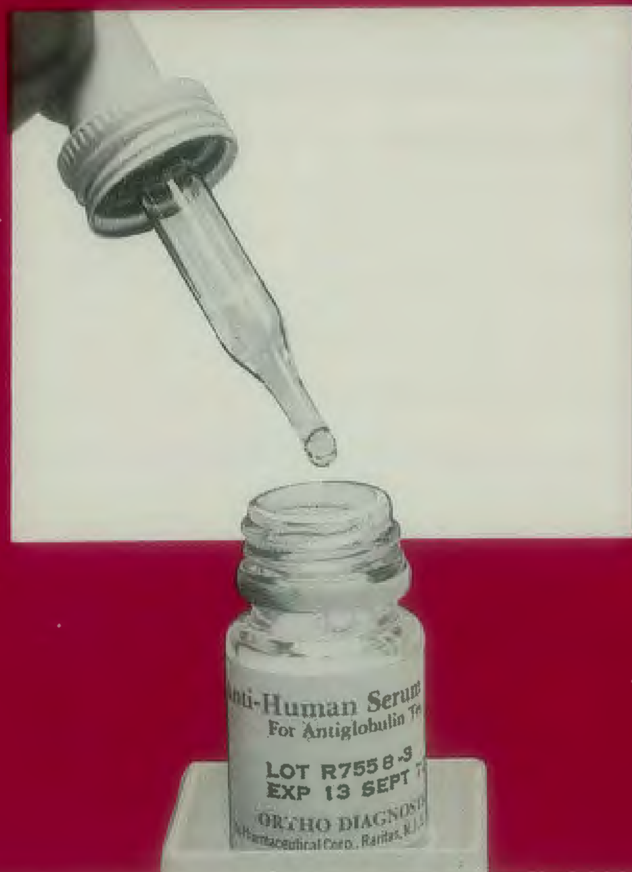

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EQUIPMENT: If a laboratory is already set up to run radioassays, there is no need for extra equipment. The essential equipment for RIA is: centrifuge, Gamma counter (or Beta counter for Tritium) and micropipetors with disposable tips.

LEARNING RIA METHODS: ALL RIA METHODS ARE ESSENTIALLY THE SAME. ONCE A TECHNICIAN IS CONFIDENT OF HIS PIPETTING OF THE RADIOACTIVE MATERIAL, HE WILL APPRECIATE THE RIA PROCEDURE.

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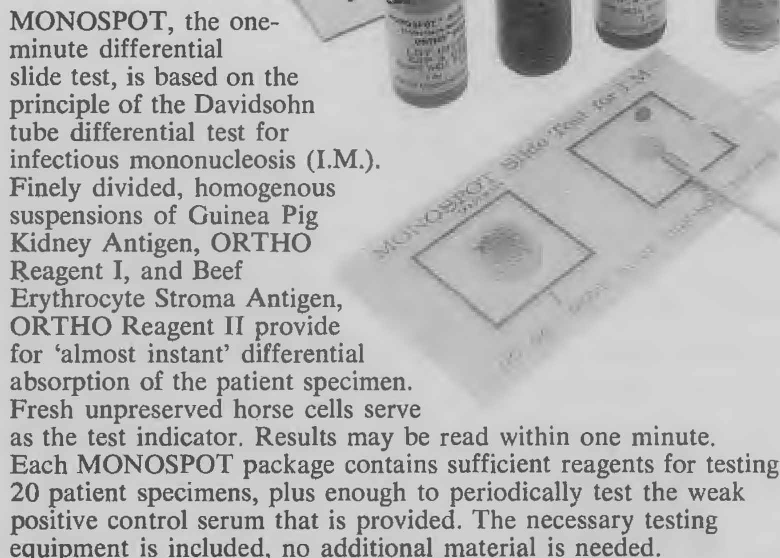

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10052

Fluids, Electrolytes and Metabolism. Robert Francis Wilson, MD, FACS. 1973. Published by Charles C. Thomas, Springfield, Illinois. 138 pages. Price, $US7.95.

This is a large practical work-book intended for reference. With this in mind it is ring-bound. It is decked out in the gold covers characteristic of the textbooks produced by this publishing house. The administration of intravenous fluids is a complex matter requiring consideration of many factors and relying heavily on laboratory results. Consequently although intended primarily for the clinician it is equally of interest to the worker in the laboratory. I found it of considerable interest providing an insight into the relevance of the estimations demanded.

This book is essentially pragmatic, providing a series of hypothetical situations, working through the problem and providing all the practical details of calculating deficits and replacements. These problems are frequently presented as questions and answers. The chapters usually start off with a concise dissertation of the principles involved and the current state of knowledge of the topic being discussed. The sections include fluid therapy in relation to hormonal effects, metabolism, renal function, electrolytes, acid-base surgery, paediatrics and burns.

The first chapter provides definitions and examples of how to calculate ionic concentrations such as calcium in calcium chloride and calcium gluconate. There are a few errors undetected in the proof reading but nothing seriously misleading. I suppose in time all these definitions will have to be recalculated in terms of SI Units.

As one might expect there are many rule-of-thumb calculations provided throughout the book. The blood volume can be assessed from the patient's surface area and the total body water can be related to the serum sodium level. Obviously experience is required in making these assessments. An observation worth quoting it that electrolyte or chemical abnormalities which develop rapidly can be corrected rapidly, but those which develop slowly should be corrected slowly.

Six pages of the chapter on metabolism are devoted to the technique of intravenous hyperalimentation including a discussion of the solutions used. This technique is increasingly used for patients with a reversible gastro-intestinal problem. Close laboratory survillance of the urine output, urine and blood glucose levels and serum electrolytes are required. One would expect osmolality measurements to be requested as well.

The physiological effects of potassium and the interaction of potassium and other ions is unravelled and finally summarised in a series of propositions. Reference is made to the 'R' fraction, that is the difference in mEq/litre between the anions and the cations which is said to remain fairly constant from day to day in a given patient. It is suggested that this may be used to check laboratory results. I have an impression that such discrepancies are rare since the advent of automated techniques in the laboratory.

Zinc is required for the proper functioning of many enzyme systems and as a low zinc syndrome is known, characterised by impaired wound healing, estimation is sometimes important.

Acid base balance produces a crop of useful approximations relating pCO_2 and pH, and the ratio of HCO_3/H_2CO_3 and pH as well as a quota of detailed examples of the actual situations which occur and how to treat them.

Flurid therapy in surgery, for cases of burns and in the field of paediatrics are similarly treated and practical instruction for specific situations again provided.

This is a field manual providing instructions for urgent situations and as such requires to adopt a dogmatic tone to impart 'standing orders'. I believe it achieves its objects in furnishing ammunition for the front-line troops and probably would not remain unthumbed on the laboratory library shelf.

R.D.A.

Basic Haematology. Arthur Simmons. Assistant Professor, Department of Pathology, College of Medicine, University of Iowa. 278 pages. Charles C. Thomas, Springfield. Illinois, USA. Price, $US12.75.

This is a book designed for medical technologists and laboratory assistants under training. It results from a series of lectures delivered by the author to students and therefore covers only the theoretical side of haematology. It is no doubt the author's intention that is should be used in conjunction with other publications describing technical methods.

The book introduces the subject at a fairly basic level covering coagulation and haemolytic anaemias as well as general haematology, there is a large section on instrumentation and a smaller chapter on quality control. Immunohaematology is not included.

The chapters on coagulation clearly outline the mechanism and role of the clotting factors, the fibrinolytic system and disorders of haemostasis. Where possible tables and charts have been used to maintain a concise presentation. In another chapter he outlines the principles of the various coagulation tests, again without any technical details.

The general haematology is well written, has been designed to be read from the front cover and not used as a reference book. It progresses through the origin, development and role of the blood cells to diseases associated with abnormal cells. Surprisingly LE cells get no mention.

Metabolism and catabolism of haemaglobin is clearly presented and the tests used for investigating haemolytic anaemias get brief but adequate coverage. The publication is marred by an occasional error (e.g., 'haptoglobins are lowered in infection') and by the use of artist's presentation of blood cells. Surely in this age of photomicrography, reproduction of genuine cells can be used.

However I would not hesitate to recommend the book for trainees to the New Zealand Certificate of Science level and for laboratory assistants working towards the examination for qualified technical assistant.

B.T.E.

Methods of Media Preparation for the Biological Sciences. Joyce A. Stewart, AB. 1974. Published by Charles Thomas, Illinois. Price, $US7.50.

The author of this book has set out to gather into one volume practical hints and as much useful information as possible relating to the preparation of bacteriological culture media. Much of this material is of the sort only gained by experience, and is not available in the text books. To a large extent she has succeeded.

In the 84 pages of text, and 19 chapters, she covers such topics as equipment, measurement of volume and pH, sterilisation, dispensing into plates, tubes and slopes, the rolling of cotton wool plugs. (A lost art!) She describes how to handle some of the more difficult media. There are sections on formulae of media, chemicals, stains and indicators, charts of chemical symbols, and common terms, A full index is included, and to finish off, ten blank pages for notes.

The style is non-technical and chatty. The four simple line drawings lend some clarity to the text. It is refreshing to read simple down-to-earth instructions, such as those suggesting the use of the side of the face to test the temperatures of melted agar prior to the addition of heat labile substances. Methods are all simple and practical, whilst the equipment described is also simple and inexpensive, with the added commendable economic use of laboratory 'bits and pieces' such as broken pipette ends, etc.

Of particular mention are the chapters on the peculiarities of different media, both standard and synthetic. In the latter is a very useful list of commonly used amino acids with notes on their solubilities and sterilisation—information I have not seen printed anywhere else. It is a pity that the list of special media discussed is not more comprehensive.

It is obvious that Joyce Stewart has had considerable experience in this field, and one can forgive the occasional dogmatic expression of a matter of opinion, and some excessively laboured points. These slight blemishes diminish the appeal of some of her statements, but do not detract from the value of the information contained within them. For the laboratory which employs unskilled labour or specialised assistants in the media making role, this unpretentious book will be invaluable. For those laboratories whose media is prepared by trainees or as part of the routine rostered work, then this book will still be a useful addition to the book shelf.

G.L.C.

Practical Laboratory Planning. W. R. Ferguson. BE (Adel.), FSADM (Arch.). Applied Science Publishers Ltd., London. Presented by the Australia and New Zealand Book Co. Pty. Ltd., Brookvale, N.S.W., Australia. 147 pages, numerous illustrations. Price, $A11.70.

The author is a laboratory design consultant formerly employed by the Commonwealth Science and Industrial Research Organisation in Australia and in writing this book gives us the benefit of some 35 years experience. The planning in this book is mainly directed

towards physics and chemistry research laboratories but the same principles of layout and services can usually be applied to other types of laboratories. The planning of the laboratories, building design, fittings, services and fire and safety precautions are all discussed in depth and detail. Mistakes always seem to occur in building, and one major miscalculation is frequently that of size. Structural problems, floor loading, vibration, elevators, windows and a myriad of other things can be adequately planned and are detailed here along with provision for future expansion as an integral part of the building.

Laboratory fittings include mention of solutions to many familiar problems. How to dispose of noxious and corrosive wastes, safe and adequate fume cupboards particularly in regard to working with perchloric acid, vibration-free benches or buffers. (Air bags are sometimes used.) The use of different materials for sinks and ducts and types of floor coverings and bench finishes are discussed.

Services occupy a large proportion of the building space in laboratories and reticulation in a false ceiling space is recommended for convenience of access. Special requirements may dictate different systems. The principle suggested for the number of service outlets is generosity without extravagance. The venting of corrosive fumes and ventilation generally, requires more thought than one might imagine, airflow around the building and prevailing winds enter into the calculations.

The material used to construct walls, ceilings and doors can be rated according to the period of times, expressed in hours that it is able to withstand standardised test conditions of temperature and loading. Large timber sections come out fairly well in these tests burning at a rate of about 1½ in depth of charcoal per hour. The effect of heat on steel girders is to cause expansion and buckling often causing collapse of buildings. The selection of material in different situations is considered and the need to exclude air by fire walls and fire breaks or baffles in vertical ducts which can act as chimneys. Automatic detection and sprinkler system have been calculated to prevent grave damage in over 99 percent of fires.

The final chapter shows pictures and plans of model laboratories in Australia, Germany and the USA.

Basic laboratory dimensions recommended in this book are those conventionally employed and provide no surprises. A 10 ft module peninsular bench unit, i.e., 5 ft bench width with 5 ft floor space; 2 ft 3 in wall bench, 3 ft height for standing and 2 ft 6 in for sitting: 9ft stud. A minimum of 180 square feet per worker. Writing spaces and knee spaces and a minimum of superstructure over the bench to collect junk. With reference to the most economical size related to structural considerations a depth of 24 ft is recommended in a laboratory with peninsular bench layout. There is a great deal of discussion on the siting and size of laboratory offices. These must relate to the function of the office and its occupants. In this book it relates to research officers seeking peace and quiet for concentration and cerebration. Clinical laboratory conditions and requirement may be somewhat different.

Two people are mainly concerned in the design and construction of a new building, the client and the architect. The client must know accurately what he wants and this necessitates preplanning with the aid of those concerned; particularly those who will be using the building. All details are required. Function, size, fittings, services, special requirements and hazards. This preplanning will involve specialists such as electricians and engineers, and the appointment of a project officer is essential. His function is to oversee all work as it proceeds and ensures that time schedules are adhered to. He will liaise between the architect and his staff, and the staff who are going to occupt the new laboratory, conveying comment and criticism. He is the 'progress man', exerting a constant gentle pressure on all parties involved. According to the author he should be like a duck; smooth and unruffled on top, but paddling like hell underneath!

In terms of the basic considerations of structure, services and planning this is an excellent book which would provide the essential advice and information in these areas. Clinical laboratories have become increasingly diverse in their function and size even within one discipline and would require individual treatment in regard to furnishing and work-flow patterns. Advice on this would have to be sought elsewhere.

R.D.A.

Medical Technology Examination Review. Volume II, Third Edition (1974). Alter, Bryan, Dittmar, Johnson, Kaminsky, Kim, Robinson, Schwartz and Wisch. 223 pages. Published by the Medical Examination Publishing Company, Inc., New York. Price, $NZ6.75. N. M. Peryer. Ltd., Christchurch.

The last edition was published in 1971 and the addition of some recent textbooks to the references and the change of one author are indications of revisions having occurred.

Chemistry contains more questions on enzymes, hormones, immunoglobulins, and lipoproteins. There are questions relating to protein-binding and radioimmunoassay, gas chromatography and automation. These all reflect current trends. The microbiology, mycology and virology questions are not markedly innovative but have been altered, replaced or recast in many instances. The same illustrations have been retained in the mycology section. Haematology and histology have been similarly treated. Immunohaematology contains some fresh material relating to hepatitis and tissue typing tests.

The format is the same as before with various types of questions: true/false, choose most appropriate answer, matching statements, and complete the sentence. Some questions require straightforward answers. The aim of the book is to help the reader check areas of weakness and to provide references for further reading. Each of the 2,000 questions is provided with an appropriate reference. This is a most convenient and time-saving arrangement but the timely warning is given that this approach is not a substitute for systematic textbook study. With the increasing emphasis on objective questions in examinations, experience can be gained by working through the multiple choice questions and gaining familiarity with the different modes of expression.

Most reviewers thought that the questions were more applicable to the NZCS level however certainly in chemical pathology the emphasis on an intelligent appreciation of the significance of the results would seem to have application at the more advanced levels. A further point made was that not all the questions or rather the answers, are in accord with policies and practises current in this country.

For the student this book is a useful adjunct to study and by supplying a great variety of questions is also a boon to the tutor.

R.D.A., D.S.F., B.W.M., H.C.W.S. and A.G.W.

Diagnostic Microbiology. A textbook for the isolation and identification of pathogenic micro-organisms. W. Robert Bailey, PhD, and Elvyn G. Scott, MS, MT (ASCP). Publisher: The C. V. Mosby Company, Saint Louis, 1974. 414 pages, illustrated. Price, $9.90. N. M. Peryer, Christchurch.

This is the fourth edition of 'Diagnostic Microbiology'. Since the first edition in 1962, there have been revisions in 1966 and 1970. Thus the authors appear to be endeavouring to maintain a re-editing programme every four years. Despite major advances in many aspects of immunology, bacterial genetics, antibiotics and medical microbiology, it is questionable whether enough information has been added to this latest edition to warrant its publication at this time. Additionally the book comes at a time when the field of microbiology and closely related subjects is being 'bombarded' with new textbooks and new editions of established texts.

However like the previous editions of 'Diagnostic Microbiology' there is a pleasing organisation of the material, with the book being divided into 11 parts and systematically covering each genera of bacteria found in medical practice. Also pleasing, is to see the latest nomenclature used for those bacteria that seem to have everlasting name changes by the taxonomists. The part covering the isolation of bacteria from various body systems and body fluids such as the urinary, gastro-intestinal and respiratory tracts or the cerebrospinal fluid and the blood, is concise and clearly presented. Two new parts, one dealing with quality control and the second by Vichazelu Iralu, PhD. MPH. on the laboratory diagnosis of intestinal parasites, are useful additions.

The book is well bound, on good quality paper, has 92 illustrations, many tables and there is an attractive coloured and appropriately designed cover.

The authors work in institutions in the State of Delaware, USA, and thus a few techniques described are those used in American laboratories. For example, the Kirby-Bauer method of antibiotic sensitivity testing takes pride of place in the antimicrobial sensitivity susceptibility section, a method not fully accepted by laboratory workers in some other countries.

The preface to the first edition of this book states that the text was designed to help at the bench of medical microbiology departments and to aid college (university, in NZ) students. As a quick reference book for the laboratory worker the book has merit but often for the more complex problems and methods required in a laboratory in 1974 more specialised and detailed works are necessary. For the student in microbiology including medical students, and laboratory technologist trainees 'Diagnostic Microbiology' would be a useful companion volume to other texts.

E.R.S.

Books Received—Reviews Pending

A Short Textbook of Medical Microbiology, Third Edition. Boards, £3.45(UK). Published by the English Universities Press, London, 1974.

Computer Technology in the Health Sciences, Shires, 1974. Price, $US10.75. Published by Charles C. Thomas, Springfield, Illinois.

Correspondence

Sir,—May I through the Journal ask for constructive comment on two practical aspects of technology which to my mind have lagged well behind other advances in the medical laboratory.

(i) Biochemical monitoring of neonates and young children. Is the traditional skin puncture for a capillary blood specimen the best or only starting point? Is the emotional trauma for a child of, say, three years of age avoidable? Is blood serum the best or only medium for routine patient assessment? Has modern technology such as dialysis anything to offer?

(ii) Advance in automation of laboratory methodology has allowed one major area to lag; I refer to centrifugation of blood specimens to separate the serum which is the starting point for most automated systems. Changes in this field during the past 30 years have been quite minor. Are there other approaches, significantly different in principle, which are being overlooked?

I would appreciate constructive discussion and consideration of these aspects of the modern scene.

D. A. McArthur,
Middlemore Hospital,
Auckland.

Abstracts

Contributors: D. G. Bolitho, Lexie Friend, J. Hannan and A. G. Wilson

CLINICAL BIOCHEMISTRY

Alpha-Antitrypsin Deficiency as an Indicator of Susceptibility to Pulmonary Disease. Diakun, R. (1974). *Med. Bull. Exxon Corp. affil. Comps.* **34**, 57.

Normal sera can inhibit 1.69 ± 0.3 g/l of trypsin, therefore standard solutions were prepared containing 10 percent (0.17 g/l), 50 percent (0.85 g/l) and 100 percent (1.7 g/l) of the trypsin usually inhibited by normal sera. Two drops of each standard were dispensed into test tubes, then two drops of fresh test serum added. Ofter 10 min incubation at room temperature, one drop of each mixture was placed on the gelatin surface of an exposed and developed x-ray plate and allowed to stand in a Petri dish for two hours at room temperature. Then digested gelatin was washed off with cold running tap water, leaving clear areas of lysis. If the trypsin had been inactived by the serum antitrypsin, the film was not cleared.

Sera from those homozygous for the gene deficiency should inhibit only the weakest standard; sera from heterozygous individuals should fail to inhibit the 100 percent standard.

J.H.

Radioimmunoassay Kits for Human Placental Lactogen. Wheeler, M. J. and Watson, D. (1974). *Ann. clin. Biochem.* **11**, 49.

There is a growing interest in the measurement of human placental lactogen (h-PL) as an indicator of placental function and foetal well-being. Several investigators have found that low levels of h-PL are frequently associated with foetal distress and may be useful in the evaluation of placental function in various obstetrical situations, including threatened abortion in early pregnancy. It has been suggested that determination of h-PL should be a routine screening test in every pregnancy.

The kits which the authors studied were: Biokit, HPL (Biolab, Belgium); Radioimmunoassay of the HCS (CEA-IRE-SORIN, France); Phadebas HCS test (Pharmacia, Sweden); HPL immunoassay kit (Radiochemical Centre, England); HPL radioimmunoassay kit (Schwartz-Mann, USA) and HCS kit (Wellcome Reagents Limited, England).

A laboratory providing a regular service for serum h-PL estimations would find it more advantageous to set up and maintain its own radioimmunoassay,

thereby assuring itself of quality control over all the reagents and standards. However, some laboratories will find it more convenient to have a reliable kit method available.

Each of the kits was studied for precision, reliability, economy, simplicity and accuracy. Satisfactory results with late pregnancy sera, obtainable within 4 h, can be achieved using the reagents supplied by Wellcome Reagents Limited or The Radiochemical Centre.

J.H.

Organ Specific Antigens in the Urine. An Indicator of Organ Damage. Rosenmann, E., Dishon, T. and Boss, J. H. (1974), *Biomed. Exp.* **20,** 1.

Normal urine and pathological urine contain a large variety of proteins emanating from diverse organs. Antoine *et al.* (1968, 1969) coined the terms histuria and histaemia for the presence of tissue antigens in the urine and in the blood.

Proteinuria is generally considered to result from impaired glomerular and/or tubular function. Exceptions are exemplified by urinary excretion of proteins of low molecular weight such as Bence-Jones protein and lysozyme. Similarly, histuria represents the contribution of tissue components to the urinary protein pool. Hence, mild proteinuria might reflect histuria rather than glomerular injury or derangement of the tubular reabsorption capacity. Use of specific antisera in urinalysis enables characterisation of these antigens in respect to their organ origin. Quantitative and qualitative alterations in the patterns of urinary excretion of tissue antigens are indictive of pathological processes affecting one organ or another. Similarly, determination of carcino-foetal antigens in the urine is of diagnostic significance in neoplastic diseases. The use of a battery of organ and 'cancer' specific antisera is advocated for diagnostic, follow-up and screening purposes.

J.H.

Micro Method for Determining Total Iron Binding Capacity by Flameless Atomic Absorption Spectrophotometry. Yu, Y. Y. and Zee, P. (1974). *Clin. Chem.* **20,** 360.

A method is described which requires only 40 μl of serum for iron and T.I.B.C. An atomic absorption spectrophotometer with a graphite furnace is used. The method is designed so that the standards used can be used for both iron and T.I.B.C. assay.

A.G.W.

Faster and Easier Radioimmunoassay of Digoxin. Drewes, P. A. and Pileggi, V. J. (1974). *Clin. Chem.* **20,** 383.

A method is described using 125 I as tracer which gives a more valid, stable, non time dependent assay. Modifications include the use of ammonium sulphate to separate bound and free portions, fresh serum pool in the standard curve, and individual specimen blanks.

The results compare favourably with the original properly controlled tritium method of Smith.

A.G.W.

Causes of Increased Plasma Creatine Kinase Activity after Surgery. Phornphutkul, K. S., Anuras, S., Koff, R. S., Seff, L. B., Mahler, D. L. and Zimmerman, H. J. (1974). *Clin. Chem.* **20,** 340.

A study was made of creatine kinase (CK) ornithine carbamyl transferase (OCT) activity after surgery to determine whether the surgery or preoperative drug treatment has any effect on the enzyme activity. It was found that halothane or succinylcholine significantly raised CK activity in the first post-operative day.

A.G.W.

Assay of Aspartate Aminotransferase Activity. Effects of Serum and Serum Proteins on Oxalacetate Decarboxylation and on Dialysis. Rej, R. and Valderlinde, R. E. (1974). *Clin. Chem.* **20,** 454.

The authors studied the effect of protein on methods measuring aspartate aminotransferase. They found that protein accelerates spontaneous decarboxylation of oxalacetate to pyruvate preventing its quantitation in assays specific for oxalacetate. Five-fold dilutions of serum in saline yielded results that were artifactually high by 56 percent. Protein increases the volumes obtained by automated procedures requiring dialysis, by increasing the amount of oxalacetate diffusing into the chromogenic flow stream. The authors suggested a diluting fluid which has a protein concentration of about 6 g/dl.

A.G.W.

Direct Microdetermination of Serum Calcium. Bayinsk, E. S., Marie, S. S., Clark, W. L. and Zak, B. (1973). *Clin. Chim. Acta* **43,** 46.

A rapid micro method for the direct determination of calcium in serum is described where cresolpthalein complexone is used as the colour reagent and diethylamine buffer to achieve increased solubility of reagents and stabilise the system.

Because of the high dilution, jaundice, haemolysis or turbidity does not appear to interfere with the system. The tests including standards and controls can be processed in less than 10 minutes.

A.G.W.

Proficiency Testing in Acid Base Analyses. An Interlaboratory Evaluation. Rej, R. and Vanderlinde, R. E. (1973). *Clin. Chim. Acta* **49,** 161.

The results of a survey of 122 New York State laboratories were evaluated. The average coefficient of variation for pH was 8.8 percent, 13.3 percent for PCo_2 and 15.0 percent for total Co_2 on serum samples. Improved performance was found in laboratories where regular quality control is used. No significant difference could be found between equipment although workload correlated with performance.

A.G.W.

A Comparison of Serum versus Heparinized Plasma for Routine Chemistry Tests. Lum, G. and Gambino, S. R. (1974). *Amer. J. clin. Pathol.* **61,** 108.

Following a long-term study on a wide range of routine tests, the authors found that heparinised plasma is preferable for electrolyte determinations and may be substituted for serum when estimating other blood constituents.

L.R.F.

Evaluation of a Direct-reading Reflectometer for Neonatal Hypoglycaemia Screening. Ente, G., Klein, S. W. and Paraswanath, B. S. (1974). *Amer. J. clin. Pathol.* **61,** 612.

An Ames Reflectance Meter using new Dextrostix reagent strips is described. This portable, simple to operate reflectance meter has been assessed over a three-year period and has proved reliable and accurate.

L.R.F.

Simplified Method for G6PD Screening Using Blood Collected on Filter Paper. Dow, Patricia A., Petteway, M. B. and Alperin, J. B. (1974). *Amer. J. clin. Pathol.* **61,** 333.

A rapid screening method for G6PD deficiency is described. It has the advantage of using blood from either a finger prick or heel prick collected onto filter paper. The method is simple, inexpensive to perform and will detect enzyme levels which are less than 25 percent of normal.

L.R.F.

Serum Copper Levels and Diphenylhydantoin. Taylor. J. D., Krahn, P. M. and Higgins, T. N. (1974). Letter to Editor. *Amer. J. clin. Pathol.* **61,** 577.

Diphenylhydantoin is a drug used for the control of epilepsy. The authors have observed that patients on this drug have significantly elevated serum copper levels and clinical biochemists should be aware of this. Studies are being undertaken to find out the interrelationships between copper, caeruloplasmin and Diphenylhydantoin levels.

L.R.F.

Direct Colorimetric Determination of Serum Calcium with O-Cresolphthalein Complexone. Morin, Leo. G. (1974). *Amer. J. clin. Pathol.* **61,** 114.

A quick and simple method for measuring serum calcium is described. The colour reagent does not have any potassium cyanide in it. The method requires only 0.020 ml of sample and is linear to 18 mg per dl of calcium. Magnesium, bilirubin, albumin, haemoglobin and phosphorus do not interfere with the estimation.

L.R.F.

Estimation of Human Chorionic Gonadotropin by Timed Agglutination. A Preliminary Report. Killip. M. (1974). *Amer. J. clin. Pathol.* **61,** 337.

A report on the use of Gonavislide, a direct-agglutination latex pregnancy test, has been made. The author has found that the rate of agglutination of the latex particles is proportional to the concentration of human chorionic gonadotropin (HCG). The results of this method compared well with the conventional haemagglutination-inhibition test procedure.

L.R.F.

HAEMATOLOGY

The Mechanism of the Entry of Dye into Neutrophils in the Nitroblue Tetrazolium (NBT) Test. Segal, A. W. and Levi, A. J. (1973). *Clin. Sci. mol. Med.* **45,** 817.

It is suggested that NBT enters neutrophils in quantities visible by light microscopy only after stimulation which produces phagocytosis of a macromolecular complex of the dye and heparin and/ or fibrinogen.

J.H.

Hairy Cell Leukemia: A Case Report. Friedman. N. H. (1974). *Conn. Med.* **38,** 342.

A recently rediscovered type of leukaemia, accounting for 2 percent of all leukaemias, is hairy cell leukaemia or leukaemic reticuloendotheliosis. This entity was identified by Ewald in 1923 and further reviewed by Gosselin (1956), Bouroncle (1958), James (1963) and Lee (1969).

Most patients will have an absolute neutropenia with 60 percent demonstrating a WBC count of $<5000/\mu l$, with up to 95 percent of the cells being abnormal. An exceptional patient will have a WBC count $>100\,000/\mu l$. The malignant cell superficially resembles a lymphoblast in size, in its cytoplasm which is finely granular or foamy, and in having a loose chromatin network. As such it is often mistaken for a normal, atypical or malignant lymphocyte. The hairy cell appears to be intermediate between a reticulum cell and a lymphocyte. The cardinal finding is the serrated periphery, made up of cilia-like projections giving the hairy appearance.

The malignant hairy cell contains a distinctive acid phosphatase isoenzyme which is tartrate resistant. This permits characteristic staining of these cells in the peripheral blood, marrow, and tissue infiltrates. This cytochemical maker is not found in lymphosarcoma, chronic lymphatic leukaemia, or reticulum cell sarcoma. Patchy rather than uniform acid phosphatase may be seen in some cells in Hodgkin's disease sections.

J.H.

MICROBIOLOGY

A Rapid Method for Determining Decarboxylase and Dihydrolase Activity. Brooks, K. and Sodeman, T. (1974). *J. clin. Path.* **27,** 148.

This paper presents an improved method for determining decarboxylase and dihydrolase activity. The medium consists of 5 grams of peptone, 3 grams yeast extract, 5 ml 0.2 bromcresol purple in 1,000 ml of distilled water with 10 grams of the appropriate amino acid added. The pH of the solution is adjusted to 5.5. The authors point out that this is the optimum pH for decarboxylase and dihydrolase activity, that glucose fermentation can be discovered by other means, that the incorporation of glucose in decarboxylase medium and that by reducing the pH to this level organisms are able to attack the amino acids immediately without first fermenting the glucose to obtain an optimal pH.

234 strains of Enterobacteriaceae and 140 nonfermentative gram negative rods were examined. It was found that the rapid method was as accurate as the Moeller method and that lysine and ornithine decarboxylase will generally be detected in 2-4 hours while arginine decarboxylase and dihydrolase required 6-8 hours as compared with overnight incubation for the Moeller broth and agar decarboxylases. The method is recommended by the authors for routine use.

D.G.B.

Toxoplasmosis—a Review. Karni, K. *Amer. J. med. Technol.* **43,** 101.

A useful review of current knowledge regarding toxoplasmosis and a brief evaluation of current tests is presented.

D.G.B.

Systematic and Rapid Identification of Gram-Negative Rods. Lakhbirsingh, and Mathews, J. T. (1974). *Amer. J. med. Technol.* **44,** 169.

A system for the identification of gram-negative rods is described. The system is devised for personnel with little experience of bacteriology, such as those employed in large departments where staff is rotated for training. The system relies on the use of a series of six initial rapid procedures carried out on commercially available rapid diagnostic reagents. From the results of the initial panel of tests further con-

firmatory test panels of biochemical tests are set up. The system merits consideration.

D.G.B

Isolation of *Neisseria meningitidis* from the Vagina and Cervix. Lewis, J. F. and Alexander, J. J. (1974). *Amer. J. clin. Path.* **61**, 216.

700 Transgrow cultures examined over a two year period by the authors showed that three of these cultures contained *Neisseria meningitidis* from either the vagina or cervix of young sexually active women. The role of these organisms in genital infection is doubtful. However, the authors stress the necessity of completely identifying all neisseria isolated from the genital tract.

D.G.B.

Comparison of the R.B. System and the Entrotube for the Identification of Enterobacteriaceae. Coppel. S. P. and Coppel, I. G. (1974). *Amer. J. clin. Path.* **61**, 218.

The authors compared these two rapid identification systems with conventional techniques for the identification of Enterobacteriaceae. There was little difference in the number of errors in the different techniques and the authors conclude that for their purposes and in their laboratory the Entrotube is an acceptable system for use. This system has recently been introduced into this country whereas to the best of this abstractors knowledge the R.B. system is not available here.

D.G.B.

Bacteraemia Due to Atypical Streptococci Belonging to Lancefield Group A. Report of Two Cases. Bannatyne, R. M. and Robson, A. (1974). *Am. J. clin. Path.* **61**, 358-360.

The authors describe two strains of Lancefield Group A streptococci isolated from blood cultures which failed to show Beta haemolysis and were resistant to bacitracin. They were also not typable by M. or T. scheme. The authors review the literature on aberrant group A streptococci and conclude that these organisms fall into two groups which are described. This paper emphasises the importance of Lancefield grouping for the correct identification of strepococci.

D.G.B.

A Clinical Evaluation of the API System for Identification of Enterobacteriaceae. Brooks, K. A., Jens, M. and Sodeman, T. M. (1974). *Amer. J. med. Technol.* **40**, 55.

This paper compares the API system with more conventional methods of identifying bacteria. The paper confirms numerous other reports in the literature that this is a reliable if somewhat expensive method of carrying out identification tests on Enterobacteriaceae. The method is well known and widely used in this country.

D.G.B.

Cotton Wool Bacteriological Swabs. Effects of Sterilization Method on Performance. Willis, L. A. and Winsley, B. E. (1974). *Med. lab. Technol.* **31**, 51.

An examination of the effects of steam ethylene oxide or gamma irradiation on the ability of cotton wool swabs to maintain the viability of bacterial inocula during storage. As the decrease in the ability of sterile swabs to maintain bacterial viability appears to be due to an increase in the acidity of the swab, incorporating a simple buffer solution before radiation sterilisation gave these swabs a superior bacteriological performance.

D.G.B.

Comparative Effects of Two Sulphated Polyanions used in Blood Culture on Anaerobic Cocci. Kocka, F. E., Arthur, E. J. and Searcy, R. L. (1974). *Amer. J. clin. Path.* **61**, 25.

The authors describe a new anticoagulant additive for blood culture. Sodium amylo sulphate which is claimed to be superior to the polyanethol sulphanate normally used in blood culture media in that it does not suppress the growth of Peptostreptococcus species in the way that polyanethol sulphate does.

D.G.B.

A Comparison by Gel Diffusion of the Lancefield and Rantz Extraction Techniques Used in Grouping Haemolytic Streptococci. Noble, R. C. and Penny, B. B. (1974). *Med. lab. Technol.* **31**, 43.

This paper compares the effectiveness of the Lancefield acid heat method of extracting carbohydrate antigens from streptococci and the simpler autoclave method of Rantz. The authors conclude that the Rantz method is a significantly better method for identifying groups B and D than Lancefield extracts and that difficulty was often experienced with Lancefield extracts of other Lancefield groups due to excessive extraction of antigen by acid extraction. This is not a problem with the Rantz method. They recommend the method for routine use. The Rantz method is of course well known and widely used already in New Zealand.

D.G.B.

The Sensitivity of *Brucella abortus* to Chemotherapeutic Agents. Robertson, L., Farrell, I. D. and Hinchliffe, P. M. (1973). *J. med. Microbiol.* **6**, 549.

An examination of the *in vitro* sensitivity of *Brucella abortus* to ampicillin, streptomycin and tetracycline, carbenicillin, gentamycin and kanamycin. The organisms were tested by a variety of methods and it was concluded that the optimum method of sensitivity testing brucella organisms is the Ditch technique. Strains were almost uniformly sensitive to tetracycline and that the 25 strains examined in this series were not as sensitive to streptomycin as organisms tested in previous surveys had been reported. A comparison of MIC plasma levels and plasma half lives suggest that gentamycin and kanamycin may be more effective than streptomycin in the treatment of brucellosis when used in combination with tetracycline. Synergism between sulphamethoxazole and trimethoprim was shown by all strains examined but the strains were less sensitive to trimethoprim than to sulphmethoxazole. This author suggests further clinical trials of cotrimoxazole.

D.G.B.

The Method of Quantitative Burn Wound Biopsy Culture and Its Routine Use in the Care of the Burned Patient. Lobel, E. C., Marvin, J. A., Heck, E. L., Curreri, P. W. and Baxter, C. R. (1974). *Amer J. clin. Path.* **61**, 20.

A method of quantitative burn wound biopsy culture is described. Full thickness biopsy specimens are obtained using a scalpel and macerated in normal saline, diluted and plated out. The results are expressed as the number of bacteria per gram of burn wound tissue. 10^4 organisms per gram of biopsy was taken as a positive finding. The authors claim that the method is a more reproducible and a more

effective aid to the early diagnosis of infection of and burned patient than surface culturing methods. The method does appear however to be extremely time consuming and the large number of aseptic operations to give every possibility of accidental contamination.

D.G.B.

Aeruginocine Typing of ***Pseudomonas aeruginosa.*** Shriniwas. (1974). *J. clin. Path.* **27**, 92.

The author suggests that the term aeruginocine typing should be used in place of pyocine typing as aeruginosa has long replaced pyocyanea as the correct specific name for *Pseudomonas aeruginosa.* Using eight indicator strains of *Pseudomonas aeruginosa* and incubating at a temperature of 32°C, this author found that there was considerable variation in the range and patterns of the aeruginocine types of strains present in various hospitals. He also found that a large number of strains isolated in India were found to fall into groups not described in the original system of Whaba's. He has therefore described the new inhibition patterns.

D.G.B.

Counting Cells in Cerebrospinal Fluid Collected Directly on Membrane Filters. Burchailo, F. and Cunningham, T. A. (1974). *J. clin. Path.* **27**, 101.

The authors present a method for the enumeration of low numbers of cells and their staining and differential counting by the use of a Swinny filter cartridge and ruled membrane filter. For those laboratories carrying out large numbers of CSF examinations this method may offer significant advantages. The authors also present a study of 291 samples which shows that the normal cerebrospinal fluid count is 2,000 cells per ml not 5,000 per ml as currently accepted.

D.G.B.

A Method of Bacteriological Sampling of Surfaces by the Direct Application of Culture Media. McCullough, B. and Cornere, B. M. (1973). *J. clin. Path.* **26**, 977.

This technical method describes a useful and economical way of carrying out surface impression cultures on agar by using disposable plastic syringes filled with nutrient medium.

D.G.B.

A Reappraisal of the Antibacterial Action of Cotrimoxazole in Vitro. Lewis, E. L., Anderson J. D. and Lacey, R. W. (1974). *J. clin. Path.* **27**, 87.

This paper questions the efficacy of the synergistic action of cotrimoxazole against urinary pathogens The results of the examination of Proteus species, other Enterobacteriaceae and *Staphylococcus aureus* is presented. The organisms were suspended in broth and in human urine to determine whether bacteriostatic synergy took place. The bactericidal action of the individual components of cotrimoxazole was also examined. The authors conclude that in many cases there is no advantage in using cotrimoxazole as trimethoprim alone is just as effective, particularly of course in infections involving sulphonamide resistant organisms.

A further consideration is that the toxicity of trimethoprim alone is considerably less than the combination of the two drugs.

D.G.B.

One Tube Oxidation Fermentation Test. Porres, J. M. and Stanyon, R. E. (1974). *Amer. J. clin. Path.* **61**, 368.

The authors describe the use of one tube of OF medium with a 10 ml fill which is stabbed to the bottom as an alternative to the usual two tube test using one 5 ml fill in each tube with an oil overlay of one tube. They conclude that the OF test is as reliable used on this single tube method as with the traditional technique.

D.G.B.

Neisseria gonorrhoeae **Identified by the Direct Fluorescent Antibody in Male Contacts.** Enfors, W., Eriksson, G., Kaaman, T. and von Krough, G. (1973). *Br. J. vener. Dis.* **49**, 500.

In this study of 167 males named as contacts of patients with gonorrhoea, the disease was diagnosed at the first examination by direct microscopy and/or culture in 60 cases (35.9 percent) but only in one additional case out of the 107 (0.9 percent) at the second visit. Examination by the direct fluorescent antibody (FA) technique of urethral material obtained after prostatic massage in 94 patients with two consecutive negative cultures revealed a further 10 cases. Only one of the 10 FA-positive patients had symptoms of urethritis.

Investigations have shown that the epidemiological significance of a small group of undetected infected individuals may be far-reaching.

J.H.

V. parahaemolyticus **Gastroenteritis: New Insight into an Old Disease.** Mackowiak, P. A. (1974). *J. La St. med. Soc.* **126**, 125.

Vibrio parahaemolyticus gastroenteritis is in all probability a very common disease. A conservative estimate is that it accounts for at least 2 percent of all foodborne outbreaks in the US. In areas such as Louisiana, where seafood is consumed in large quantities, it must occur with a substantially higher frequency.

The organism is a motile Gram-negative bacillus [curved when grown under optimal conditions] possessing a single polar flagellum, the natural habitat being estuarine waters and underlying mud in most areas of the world. A unique feature of the organism as well as the reason for its years of anonymity is its salt requirement. As a halophile, it will not be isolated by routine stool culture. A salt-enriched medium such as thiosulphate-citrate-bile salt sucrose (TCBS) is required.

Strains of this organism are currently classified into 10 O antigen groups and 52 K antigen types. Approximately 10 percent of the strains are not typable. Pathogenic strains can be distinguished by the ability to produce haemolysis on salt-enriched blood agar (positive Kanagawa reaction).

This type of gastroenteritis is a relatively mild disease although fluid and electrolyte imbalance resulting from the illness may pose a serious threat to otherwise debilitated patients. Secondary person-to-person spread does not appear to be an important factor in the epidemiology.

J.H.

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