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Laboratory Workload Recording System for use in Manpower Planning

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Received for publication, October 1974

1. Introduction

With health costs rising it is incumbent upon all responsible managers to attempt to achieve increased productivity and to use objective arguments for manpower planning. When it is realised that about 80 percent of the money spent on the hospital system is in salaries and wages and also that the hospital system is making a disproportionate demand on the labour market (see Table I) the inevitability of tighter controls becomes obvious.

TABLE 1

Year	% Increase in Labour Force	% Increase in Hospital Board Staff
1967	2.6	6.2
1968	1.7	5.1
1969	3.4	10.2
1970	6.3	13.8
1971	—	—
1972	1.0	5.9
1973	2.4	5.2

(The gaps are caused by changes of definitions in the compilation of Hospital Board Staff statistics).

Staff increases are often argued on subjective, and sometimes highly emotional grounds. Certainly there is very little effort to plan manpower requirements.

When attempts have been made at manpower planning there is often a suspicion on the part of the departmental head (particularly if the planning is carried out by clerical administrators) that his " professional judgement " is being questioned. This is probably caused by the departmental head on the one hand failing to appreciate the limitations of productivity calculations as a basis to planning and on the other hand the failure of the clerical administrator to appreciate the need to maintain or improve in-depth quality of service.

Ideally any system should take into account both productivity and quality of service.

As part of the planning base for the management system used in this laboratory, the following method of manpower planning based on workload units to derive productivity and a staff quality index has been developed.

2. Workload Units

The College of American Pathologists and the Canadian Association of Pathologists have carried out time engineered studies to determine workload unit values for most laboratory tests. These studies have been carried out in a number of laboratories using both manual and machine techniques.

The unit values are obtained on the basis of the average technical and clerical time needed to accomplish each procedure. Each unit is equivalent to one minute of technical and/or clerical time, i.e., one unit is equivalent to one minute and five units to five minutes. The factors assigned to a test include the time for all activities that occur within the laboratory in order to complete that test once.

The activities are

(a) Initial handling of the specimen

Initial handling includes all the steps from the receipt of the specimen by the laboratory to the completion of all preliminary preparation and recording required before testing can be started. Initial handling will include time stamping the requisition, sorting specimens, recording the patient's name, assigning a laboratory number, entering information on a worksheet, labelling the samples, centrifuging and separating the serum and delivery to the work area.

(b) Specimen testing

Specimen testing includes all the technical steps required to perform the laboratory test up to and including the recording of the result.

(c) Recording and reporting

Recording and reporting include all the steps required to report the result. Recording and reporting will include recording a result on the patient's report and in the laboratory records, the checking, sorting, filing and sending of final reports.

(d) Technical supervision

Technical supervision includes the technologist time required to directly supervise the test procedure. In this area activities such as checking quality control results and approving the reporting of results are to be included. Other factors which are included are daily preparation, e.g., instrument calibration, maintenance and repair, solution preparation, glassware washup.

Some work has been done by H. W. Taylor[2] to compare New Zealand workload units with the Canadians and it would appear that the Canadian Schedule of unit values[3] would suit local conditions.

3. Method of Calculating Productivity

The method of calculating the productivity values depends upon the collection of the total numbers of each test type and their multiplication by the various test unit values. This gives a total of workload units. The workload units are then divided by the number of worked hours and a productivity value is derived which is a measure in minutes per hour of the time worked, e.g.,

Workload units equals 50,000.
Worked hours equals 1,000.
Productivity equals 50 minutes per hour.

The productivity values are calculated on the basis of minutes per hour worked and the theoretical maximum for an individual and a laboratory is 60 minutes per hour. However, there are certain factors which for the purpose of practical productivity can be classed as idle time.

4. Idle Time and Practical Maximum Productivity

Idle time can be briefly defined as that time when productivity is either non-existent or minimal. Idle time can be derived from four main sources:—(1) Lecture attendance; (2) Annual leave; (3) Sick leave; (4) Refreshment breaks.

It is necessary to quantify idle time because it obviously must be taken into account when the practical maximum productivity value is calculated.

Calculation of Idle Time

There are variable amounts of lecture time depending on the type of worker, i.e., trainee technologists differ from laboratory assistants. Categories of workers can be classified as pre-New Zealand Certificate of Science (NZCS) and others.

Pre-NZCS students claim about 11.5 hours per week equals 20 percent of worked hours. Others claim about 2 percent of worked hours (the average needs to be calculated taking into account the mix of staff). Refreshment breaks claim 2.5 hours per week equals 7 percent of worked hours. Annual and sick leave claim (say three weeks plus one week) equals 7 percent of worked hours. These figures will vary for different laboratories.

The calculated figure is subtracted from the theoretical maximum of 60 minutes per hour to derive the practical maximum productivity, e.g.:

(i)	Lecture attendance: Average	= 9%
(ii)	Refreshment breaks	= 7%
(iii)	Annual and Sick Leave	= 7%
	Total idle time	= 23% = 14 minutes
	Practical maximum productivity	= 60 mph minus 14 mph = 46 mph

To make an allowance then for idle time we could have the following calculation:

Workload units	40,000
Worked hours	1,000
Productivity	40 min./hr
Idle time	14 min./hr
Practical	$\frac{40}{46} \times \frac{100}{1}$
Maximum Productivity Percentage	= 87%

5. Relationship of Productivity to Quality

As a basis for planning the calculation leaves much to be desired. Productivity alone describes the result of work. It is the end product of a complex of activities carried out

by a number of people. It does not take into account the quality of the end product.

It is not easy to measure the quality of the end product of laboratory operation. Quality control programmes will of course assist but they are not the complete answer.

There is often scepticism of applying mathematics to staffing needs and this factor of quality is one that is most hotly argued. Certain aspects of laboratory work require skills acquired from years of experience and training. Such skills are usually described in emotive terms but they are nevertheless very real factors to be taken into account when discussing productivity.

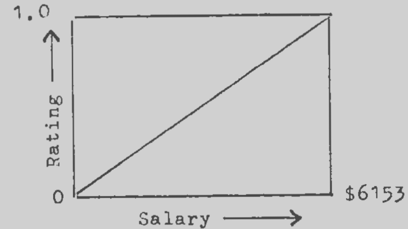
These factors are always difficult to argue because of the inherent difficulties in measuring them. If however, they are quantifiable they should be included in any mathematical approach to manpower planning.

A factor has been built into the productivity calculation to allow for quality based on our most valuable asset — people. To measure objectively the quality of work it is worth noting this statement from Elliott Jaques[4] "... two sets of criteria must be met. First, the normal conditions of any scientific definition must be served. The definition must be objective in the sense that what is defined can be observed by anyone who follows the definition, it must be both comprehensive and exclusive, in the sense of identifying what it is intended to refer to and excluding the rest of the field, and it must serve as a basis to quantification, for if a definition is objective, then the amount of whatever is so defined ought to be open to measurement. Second, the definition must encompass the notion of responsibility. For no sooner do we consider the question of relative value of different types of work in terms of payment differentials than we find ourselves in the field of argument about the relative degrees of responsibility in the various types of work under consideration."

A method of applying such a measurement is discussed here which is based on applying ratings to workers based on academic achievement and salary.

6. Calculation of Staff Quality Index (SQI)

A decision has been made based on professional judgment to award the highest rating to a bench worker who is the holder of the Certificate of Proficiency in Medical Laboratory Technology (COP) with a Part III qualification in a particular discipline. A worker must have spent one month in a department to gain a rating. The COP holder has a rating of 1.0 percent and to derive the ratings of other workers, a simple graph has been drawn relating salaries to ratings.



Example of workers' ratings would be:

COP	1.0
Basic Training Certificate	0.92
Second year trainee	0.5
Qualified Technical Assistant with University Entrance	0.6
Qualified Technical Officer with University Entrance	0.8

Examples of Calculating the SQI

STAFF	INDEX
5 at $6153	5 × 1 = 5
4 at $3066	4 × 0.5 = 2
5 at $2716	5 × 0.44 = 2.20
14	9.2

$$S.Q.I. = \frac{9.2}{14} = 0.66$$

Example of How to Adjust the Mix of Staff to Achieve the Same SQI

STAFF	INDEX
5 at $6153	5 × 1 = 5
2 at $5672	2 × 0.93 = 1.85
2 at $3416	2 × 0.55 = 1.1
4 at $3066	4 × 0.5 = 2.0
8 at $2716	8 × 0.43 = 3.44
21	13.40

$$S.Q.I. = \frac{13.4}{21} = 0.64$$

7. Other Factors to be Accounted for in Manpower Planning

(a) Fluctuating Workloads

It is acknowledged that there are factors outside the control of the laboratory which cause fluctuations in workloads. These factors can be partially controlled but for planning purposes their effect can only be minimised by averaging workloads.

(b) Training Programmes

These are compensated for in Idle Time.

(c) The Planning and Staffing of Special Units

It is acknowledged that a case may be made from time to time for an increase in establishment on the grounds of the setting up of a special unit within a department. The usual argument for this is based on the development of a special area of work where an initial staffing may be in the ratio of one supervisor to one worker.

Productivity measurements in these areas cannot initially be used as a basis for staff planning.

8. Calculation of Productivity Percentage

Having identified the various factors which can be measured, the calculation for productivity becomes:

$$\text{Productivity \%} = \frac{\text{Productivity}}{(60 - \text{Idle Time})\ \text{S.Q.I.}} \times \frac{100}{1}$$

Examples will be given of its use.

(a)

Workload units = 120,000
Worked hours = 4,000
Productivity = 30 min./hr
Idle Time = 14 min./hr
Effective Time = 46 min./hr
Staff Quality Index = 0.6
Practical Max. Productivity = 46 × 0.6 = 27.6 mph

$$\text{Productivity \%} = \frac{30}{27.6} \times \frac{100}{1} = 109\%$$

This figure probably represents an overworked situation with consequent loss of quality.

(b) If more highly qualified staff are employed in example (a) the percentage returns to a more realistic level.

Workload units = 120,000
Worked hours = 4,000
Productivity = 30 min./hr
Idle Time = 14 min./hr
Effective Time = 46 min./hr
Staff Quality Index = 0.7
Practical Max. Productivity = 46 × 0.7 = 32.2 mph

$$\text{Productivity \%} = \frac{30}{32.2} \times \frac{100}{1} = 93\%$$

9. Utilisation of Data

(a) Staff Quality Index

Obviously an increase in senior trained staff will alter the SQI.

Total staffing is complicated by the need on one hand to provide more trained staff whilst on the other hand striking a balance by taking on trainees to eventually provide the trained staff and further by appointing laboratory assistants to provide stability to enable trainees to train. Proposed alterations to the training programme which utilise full time attendance at technical institutes rather than day release should change the situation.

Other problems which complicate staffing are not unique to laboratories such as, for example, 24-hour staffed rosters and fluctuating workloads. However, the primary objective to be aimed for, taking into account the variables is the employment of as few highly productive adequately trained and experienced workers as is necessary to manage the workload.

(b) Idle Time

A reduction in idle time can only be taken so far. In particular it is affected by the amount of time required for training programmes.

(c) Workload Units

Workload units are obviously related to the amount of work and so are used in the planning operation. It is a controllable variable in so much as the total units may be altered by purchase of a machine to perform tests previously carried out manually or with considerably slower machinery. The argument for purchasing such a machine is often well supported but could be more powerfully applied by use of productivity data. In example 1 it can be seen that before purchase of a 12-channel analyser, staff were working considerably in excess of the practical maximum with probable attendant problems associated with quality. Following the purchase of the new machine the level of productivity was reduced to an acceptable level.

The supporting data can also be used to demonstrate how purchase of such a machine could alter total throughput of the laboratory by making a second shift possible without the considerable expense of a heavy manpower commitment.

Example 2 is shown where a department may have already mechanised its basic routine testing but finds its practical productivity being exceeded by an individual test manually performed and increasing in number. Such a test could be platelet counting and in the example it is demonstrated how the throughput could be increased with a machine.

Example 1

To demonstrate changes in practical productivity following the purchase of a 12-channel analyser. Work was previously performed on 2- and 4-channel machines:—

	Before Purchase	*After Purchase*
Worked units	135,000	100,000
Worked hours	4.500	4.500
SQI	0.5	0.5
Effective Time	46 min./hr	46 min./hr
Practical Max. Productivity	23 min./hr	23 min./hr
Productivity	30 min./hr	22 min/hr
Productivity Percentage	130%	96%

It could be argued that the productivity percentage after purchase has resulted in such a change that some staff should be declared redundant. This type of reasoning fails to take into account either failure on the part of the laboratory to argue for purchase of such a machine (taking into account rising workloads) until after staff have been employed to manage, however, inadequately, the rising workload, or failure by hospital administrators to take notice of the laboratory administrators' attempts to argue for such a machine. This highlights the dilemma of both sides in a planning operation.

Example 2

To demonstrate change in productivity resulting from the purchase of a machine for platelet counting to replace a manual method.

	Before Purchase	*After Purchase*
No. of platelet counts	2,000 per month	2,000 per mth
Workload units	10 × 2000 = 20,000	2 × 2000 = 4000
Total workload units for all tests	130,000	114,000
% of units attributed to platelet counts	15%	3%
Effective time	46 min./hr	46 min./hr
Time attributed to platelet counts	6.9 min./hr	1.4 min./hr

10. Conclusions

Laboratory services are steadily expanding, and like other sections of the hospital service are often because of pressure of work, caught up in the problems associated with unplanned expansion. In particular, one of these problem areas is that of manpower planning. It is not suggested that the method described in this paper is foolproof but it is suggested that it at least provides some scientific basis to general planning which cannot help but be an improvement on present methods.

One of the major criticisms could be levelled at the staff quality index. In the absence of any more sophisticated method of making an allowance for quality, it seems reasonable to relate this to theoretical and practical training which in turn is salary related.

One of the problems in laboratory manpower planning is associated with projecting workloads. This also creates difficulties in laboratory budgeting. It is suggested that there is a need to study the laboratory requesting patterns of the various consultant teams and areas within a hospital to derive sufficient information for a detailed investigation of this problem.

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Addendum. As an example of a real life situation the following worked example is presented.

The problem relates to the fact that there are two large hospital laboratories within 400 metres of each other. The haematology department of laboratory A has a Coulter "S" counter, laboratory B has not. A policy has been decided upon of centralising the haematology services for the combined

hospitals in laboratory B. The question is how many staff will be required to maintain the centralised service.

Productivity calculations for hospital A

Total workload units for a 3-month period = 159,060

Staffing — 2 Graded Officers
2 Staff Technologists
2 Basic training certificate holders
8 Trainees
2 Laboratory assistants

Total	16		
Total hours worked (excluding Graded Officers)	=	7,280	
Productivity	=	22	minutes per hour
Idle time	=	13	minutes per hour
Practical max. Productivity	=	47	minutes per hour
Staff Quality Index	=	0.65	
Productivity %	=	72 %	

Productivity calculations for Laboratory B

Total workload units for a 3-month period = 298,353

Staffing — 2 Graded Officers
3 Staff Technologists
3 Basic training certificate holders
8 Trainees
7 Laboratory assistants

Total	23		
Total hours worked (excluding Graded Officers)	=	10,920	
Productivity	=	27	minutes per hour
Idle time	=	12	minutes per hour
Practical max. productivity	=	48	minutes per hour
Staff Quality Index	=	0.64	
Productivity %	=	87 %	

Specimen Collection for Laboratory B

Average number of specimens collected per weekday equals 122.

Based on an estimate that 95 percent of specimens are venepunctures and 5 percent are micro collects and 95 percent of the total number of specimens are collected by laboratory collecting staff the following staff would be required:

Total Venepuncures 109 × workload units 8 = 872
Total micro collects 6 × workload units 12 = 72
Total workload units = 944

If 2.5 full time equivalent staff are used for specimen collection:

Total units per 5 day week	=	944 × 5 = 4720
Total hours worked per week	=	120
Productivity	=	$\frac{4720}{120}$ = 39 min. per hour
Idle time	=	8 minutes per hour
Practical max. productivity	=	52 minutes per hour
Staff Quality Index	=	1.0
Productivity %	=	75%

Specimen Collection for Laboratory A

Average number of specimens collected per week day equals 130.

By comparison with laboratory B the specimen collection figures are very similar and no further details need be calculated.

Discussion

If the present workloads were combined and carried out on the Coulter "S" counter using combined staff, the productivity percentage would be unacceptably low at about 65 percent. It is obvious that fewer technical staff would be required. On the specimen collection side, although 2.5 full-time equivalent staff in each hospital only have about 75 percent productivity the collection of samples for other departments plus workload expansion would probably take up the slack.

To achieve a 97 percent productivity with the workload combined in laboratory B and performed on the Coulter "S" counter and allowing for a 15 percent increase in work in the first 12 months the following staff would be required:

4 Graded officers.
6 Staff technologists.
6 Basic training certificate holders.
6 Trainees.
6 Laboratory assistants.
5 Full-time equivalent blood collectors (2.5 in each hospital).

Cost

The present combined salaries of the haematology departments of hospitals A and B is $185,500 per annum.

The suggested staffing for a combined department in hospital B totals $159,500 per annum.

Some Comments on the Education of Medical Laboratory Technologists in New Zealand

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Background

The Examination System:

Medical Technology as an examinable subject probably goes back some 30 years. The first examinations were organised on an informal basis and have always consisted of theory papers, practical and oral examinations. The first change in the five-year C.O.P. system was the introduction in October 1949 of an Intermediate examination taken after three years.

This system of examination remained in use until 1966 when the first of the Single Subject Certificate of Proficiency Examinations was held. On May 8, 1964, the Medical Laboratory Technologists' Board was set up and after a number of meetings decided on further changes. These changes were brought about by:

(a) The recognition of disciplines within the broad field of Medical Laboratory Technology.

(b) The subsequent pressure to produce specialists.

(c) The realisation that the syllabus content of the general Certificate of Proficiency was becoming too large to be manageable, both for teaching and examining purposes.

(d) The very large number of students presenting for the Intermediate Examination made practical examinations at this level impossible to conduct.

The changes proposed as a result were,

(a) Removal of the practical from the Intermediate Examination and its replacement with a work-book assignment scheme. Theory and oral examinations were retained and the qualification became known as the Basic Training Certificate.

(b) Preparation of syllabuses for examinations at two levels to be known as O and A in each of Bacteriology, Haematology, Biochemistry and later Histology/Cytology.

(c) The decision to allow two routes to the attainment of the Certificate of Proficiency, either by taking two O levels or an A level.

It should be noted that this change was a compromise in that whilst recognising the emergence of specialties, the examination system was still geared to producing a partially trained general person. Indeed the A Level examinations were stated not to be specialist ones but only an advance on the O Level.

Like most compromises the arrangements were not entirely satisfactory and this is best highlighted by the very arbitrary division of syllabus material into O and A Level and the subsequent difficulties for students, tutors and examiners in deciding where one level began and the other one ended. This problem is still with us some ten years later.

The new system did however recognise the emergence of specialties and to this extent a major policy decision had been made in splitting up the general Certificate of Proficiency. In the intervening ten years the operations of the Medical Technologists' Board and more recently the Medical Technologists' Registration Board have been machinery ones. Syllabuses have been reviewed from time to time, the practicalities of the examination system have been tended to and improved but no major policy decisions have been made.

The administrators of this system have tended to follow rather than to lead. This criticism is based on the following observations:

(a) If the emergence of specialties was recognised in 1964 why have specialist examinations not been offered and the various problems associated with the arbitrary syllabus decision not solved?

(b) Why, apart from immunohaematology (and that only recently) have not other disciplines been added to the available examinable subjects?

These criticisms assume serious proportions when one considers the intervening period of stagnation which has resulted in:

(1) The emergence of nuclear medicine and its disappearance from medical laboratories.

(2) The emergence of medical cytology, the formation of its own society and its almost complete utilisation of technical assistants.

(3) The dearth of suitably trained histology technologists.

(4) The heavy utilisation of technical assistants in immunohaematology.

These examples should be sufficient to emphasise the fact that the true emergence of specialties is long overdue.

The Training System

The training system for medical laboratory technologists was originally based on an apprenticeship-type system. That is to say, students learnt the skills by working on the bench alongside workers who if not qualified were at least usually more senior in terms of work experience. Theoretical training was carried out by a series of lectures given by qualified staff. Students were moved at regular intervals through the various departments of the laboratory to acquire the necessary general training.

This system, although somewhat haphazard, worked well enough, mainly because of the high qualified staff/student ratio. In fact it could well be argued that this type of training especially from the point of view of acquiring practical skills is still the best. However, several factors led to a breakdown in the system, the main contributing one being the very rapid build-up of work in the late 1940's and early 1950's. As expected the metropolitan centres were the first to feel the effect of this work increase. The impetus to laboratory medicine at this period probably resulted from a return to peace time conditions, the carry-over into medicine of many of the mass testing techniques developed during the war and the development of the Social Security system.

In the laboratory situation, this increase in demand for laboratory facilities resulted in the setting up of private laboratories, a drain of qualified staff away from the hospitals, and the subsequent destruction of the high qualified staff/student ratio. The high ratio was even further eroded by the necessity to bring in more laboratory students to manage the rising work loads, and to act as a stop-gap in the drain on qualified staff away from hospitals.

The apprenticeship system of training was at an end.

The Medical Laboratory Technologists' Board in its early days noted the problem of training and took the positive step of recognising the need for tutors. Over a period of time tutors were appointed in metropolitan areas and education was organised on a more formal basis, at least for the theoretical aspects of the course. These courses were all organised by local tutors and a large part of the country was embraced by this upsurge in teaching.

The practical aspects of teaching remained however to be passed on by students "rubbing shoulders" at the bench with workers only slightly senior to themselves.

The next major change and perhaps the most profound since the emergence of medical laboratory technology was the decision to move the education system into the technical institutes via the N.Z. Certificate in Science. The modified New Zealand Certificate in Science course was duly formulated and was to replace the Basic Training Certificate examination, although the Medical Laboratory Technologists' Board retained the right to award the Basic Training Certificate to holders of the New Zealand Certificate in science (para-medical), who had attained suitable practical experience.

The NZCS course is now in its fifth year and embraces the whole country either through block courses at the Central Institute of Technology or by day release at local technical institutes.

Like many such changes the Technicians' Certification Authority course has brought its share of disappointments, recriminations and rewards but the decision to move in this direction represents a major breakthrough in that it recognised the need to place the theoretical and practical aspects of training at least to the

Basic Training Certificate level on an organised basis within the framework of a tertiary teaching institution.

By so doing the Medical Laboratory Technologists' Board formally recognised that the haphazard apprenticeship system of training had ended.

Technical Assistants

The changing staffing patterns of the laboratory, with, in particular, the upsurge in the numbers of technical assistants is not a New Zealand phenomenon. Technical aides and other types of laboratory workers, not undertaking the technology training programme have been employed in increasing numbers overseas.

The first attempts to use technical assistants on any large scale (laboratory assistants have always been used in laboratories particularly in areas such as histology, museums, etc.) was in 1957 when the Director of Laboratory Services at Auckland Hospital permitted the Blood Bank to be mainly staffed with this class of worker. In 1959 the same director experimented with the staffing of almost the entire laboratory at National Women's Hospital solely with technical assistants.

Since that time there is hardly a laboratory in the country, both public and private that does not employ technical assistants in one or more of its departments. It could even be argued that many labs would not function without the employment of substantial numbers of these people.

One disturbing feature however of the whole system is the employment of such people in large numbers in areas where the medical technology course has not catered, e.g., cytology. Indeed there are some positions currently held by technical assistants that should probably be filled by technologists. This is not to say that many of these senior technical assistants particularly those holding the Qualified Technical Officer Certificate, are not well trained, experienced workers. The fact is that many of them would have made excellent technologists if they had had the opportunity to train.

The New Zealand Institute of Medical Laboratory Technology very wisely at an early stage saw the need to take these people under its wing and not to alienate them and so the QTA and QTO system of examinations already in use in Auckland Hospital Board's laboratories was taken over by the Institute.

As one cannot discuss a total educational system without reference to technical assistants it is worth stating the reasons for the upsurge in the use of this class of laboratory worker:

(1) The difficulties of rostering trainees through departments, sometimes at very frequent intervals, caused considerable problems. Every change resulted in a considerable staff upheaval with consequent deterioration in standards. This was probably the main reason for seeking staff stability in blood banks with technical assistants.

(2) The more recent upheavals due to day release have reinforced the necessity for a core of stable staff to allow any department the luxury of technology training at technical institutes.

(3) The difficulties of recruiting, training and holding staff in the very small laboratories.

(4) The economics of the training system, in particular, the high post NZCS salary have caused most private laboratories to give up training.

(5) Finding that a good calibre of person could be recruited and held on to by means of the examination system has lent support to the argument that technical assistants are often as good, if not better, than the calibre of person recruited to the trainee ranks.

No system of education developed for the future can fail to take into account these factors.

University Graduates

University graduates, like laboratory assistants have always been employed in medical laboratories. Usually they have been found in the chemistry areas although more recently those with specialist degrees have found their way into other departments, e.g., virology, bacteriology.

They are still employed in small numbers as noted in the recent Bolitho (1974)[1] staff survey. Some departments in some hospitals have made greater use of them than others, this being related to a particular staffing policy

of that department. However, generally speaking this is an exception to the rule. Some private laboratories have also employed university graduates in larger numbers as a cheaper source of labour than technologists.

One change that has taken place is the reduction in the number of university graduates entering the COP course. The main reasons for this is probably the disenchantment of employers with university graduates' ability to cope with the COP course. This is not surprising for by comparison with the technology student the university graduate is very short on practical experience.

University graduates have a role to play in the total staffing structure of laboratories. In particular their basic science training in chemistry, physics and the biological sciences fit them in particular for developmental work. Any educational developments should attempt to incorporate rather than isolate and alienate them.

Problems Associated with the Present Training Course

The New Zealand Certificate in Science

The present course is based on day release and block courses. Both create problems for students and for laboratory managers. A list of these could include:

(a) Problems of concentration at night classes after a day's work.

(b) Problems associated with travelling to and from work to classes.

(c) The almost complete lack of association with what is being taught at the technical institute with what is being learnt at the laboratory work bench.

(d) Pressures associated with intensive study at block courses.

(e) The lack of relevance of the basic science part of the course to medical laboratory technology.

(f) Problems for managers and students of rostering to provide a twenty-four hour coverage.

Part 2 and 3 Levels

Some of these problems have already been highlighted in particular those associated with the division of disciplines into parts 2 and 3. In addition the following could be noted:

(a) The haphazard and disorganised teaching leading to the examinations.

(b) Laboratory manager's rostering problems with two streams of people taking examinations.

(c) The complicated and expensive examination system.

(d) The lack of a real list of training laboratories for the two levels.

(e) Failure to produce true specialists.

Professional Problems

The changes which have occurred over the last several years have placed us in a position to claim professional responsibility. The recent setting up of a State Registration Board is an obvious one. Another is the shortage of pathologists, particularly in the hospitals which does not appear to be improving and this has caused many of our people to be thrust into positions of responsibility.

One is also loath to separate by definition the various categories of laboratory workers and in particular technologists from technicians. And yet an attempt should be made to do this if only because the whole future of medical technology is inextricably bound up with the creation of a professional image. It has been argued from time to time, and this more often by people who have already attained imminent standing within their own profession, that qualifications do not matter so much as good practical experience. I accept this argument to a point but until the whole structure of education and society accepts this, standing in the community and the associated rewards and responsibilities will continue to be judged on the attainment of academic qualifications and the creation of an image of professionalism. To relate this philosophy to our own situation I will stick my neck out and define the elite of our profession as technologists and the next class of worker down as technicians.

To follow this argument through, it seems desirable to produce a system of education which:

(a) is open ended in that it allows people depending on their circumstances, motivation and abilities to proceed to various levels.

(b) produces sufficient numbers of a technician workforce to manage the workloads in all laboratories.

(c) produces sufficient numbers of technologists to lead the profession.

It is the technician group who should provide the major work force in most laboratories. This would be the generally trained worker for small and medium sized laboratories and it is from this group that replacements for technical assistants should come. If the profession is to acquire this professional image then it must not only look to its top echelon but must also look to its lower echelon. The fact that technical assistants are employed in such large numbers at the moment is because the training programme does not meet the current needs. As stated earlier, many technologist positions are currently filled with technical assistants. The profession cannot afford such a large group of relatively untrained people and if the technical assistant group is to be put into perspective more technicians should be produced.

At the other end of the scale we have diluted the profession by engendering the attitude that " one is not qualified unless one has a COP ". In fact the word " qualified " should be discarded and the attitude adopted that one has reached a certain level of training which is marked by the passing of examinations which allows one to apply for certain types of positions.

Types of Laboratories

It is always difficult to project the likely development in an area of expanding technology, yet, an attempt must be made if some rationalisation is to take place in the education field.

Properly used the lessons drawn from historical development can be used as guidelines. One such historical fact is that the proper development of medical technology has often been hampered by parochial interest. The metropolitan laboratories with their problems trying to force issues which have not been acceptable to medium sized centres and sometimes the smaller laboratory representatives have dictated the policy.

Whilst it is proper that the total New Zealand scene should be taken into account, it is inevitable that metropolitan centres will face problems associated with their growth before other centres. Provided solutions to their problems fit into an orderly overall scheme there is no reason why more than one system should not be operating. *There should be positive acceptance of this principle.*

Types of Laboratories

A series of activities can be envisaged which when put together in various combinations make up a model representing the various types of laboratories. Such activities could be:

(1) Development.
(2) Manual Testing.
(3) Mechanised Testing.
(4) Automated Procedures.
(5) Quality Control.

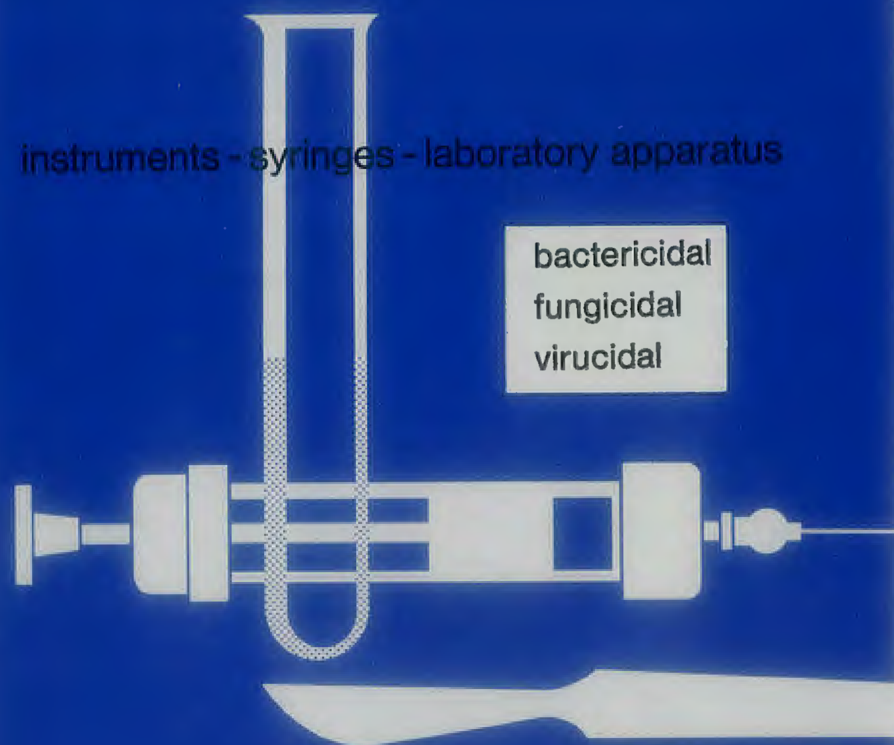
In tabular form these activities could be represented as:

Type of Laboratory	Development	Departmentalisation	Emerging Specialities	Mechanisation	Automation	Range of Test
Large	†††	†††	†††	†††	†††	†††
Medium	†	†††	–	†	–	††
Small	–	–	–	–	–	†

Conclusions which may be drawn from this section indicate that projected staff requirements based on the types of laboratories operating in New Zealand show considerable variation. At the one extreme there are a few very small laboratories offering a good general service with restricted training facilities. At the other extreme are a few very large highly departmentalised laboratories, often operating in conjunction with medical or clinical schools of medicine. The latter have good training facilities and offer a very wide range of tests, many of them performed by machines.

It would appear that the best means of satisfying all requirements would be to provide a training scheme which has the following specifications:

(1) A basic science content to provide a base for continuing education.
(2) Produces some general practitioners.
(3) Produces some specialists.
(4) Defines the requirements for recognition of a laboratory as a training centre.



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Training Programme

As a basis for suggesting a training programme the requirements and conclusions extracted from the previous statements should be listed.

(1) True specialists are required now.

(2) The range of specialities offered should be expanded to cater for existing and emerging disciplines.

(3) Day release, block courses and the apprenticeship type system present difficulties for both students and managers.

(4) Generally trained workers are necessary for small laboratories and some medium size laboratories.

(5) Small laboratories are not suitable for training.

(6) University graduates should be encouraged into some areas of the laboratory.

(7) University graduates not working as specialists should be encouraged into the training programme for technologists.

(8) Technical assistants are a vital part of the present work force but the employment of them in such large numbers is open to question.

(9) Any system of training must be open ended.

Full-Time Courses

One obvious solution to the problem of day release and block courses is to set up full-time courses. The disadvantage of full-time courses are:

(1) No guarantee of employment once the course is completed.

(2) Graduates from the course are short of practical experience.

(3) No guarantee that the present appeal to school leavers to enter medical technology will be maintained.

Most of the disadvantages are probably surmountable if close liaison is maintained between the teaching institution and the local medical laboratories. In fact whatever the course structure one could say that based upon the effectiveness of this relationship, so the course will either succeed or fail.

The basis of the full-time course should be the provision of sufficient basic sciences leading into medical laboratory technology 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 any routine techniques in any laboratory. The academic level would need to reach probably about the present Part II level.

The course could be of four years' duration and would comprise two years full time in the teaching institution and two years in a recognised training laboratory with 1,000 hours in each of haematology, clinical chemistry, bacteriology and immunohaematology, with a minimum of 160 hours of cross-matching. Students completing this course would obtain the Basic Training Certificate in Medical Laboratory Technology and would apply for positions and would be eligible for state registration. Workers so qualified could be called technicians.

The course could be taught at technical institutes on behalf of the Medical Technologists' Registration Board. The course would no longer be a Technician Certification Authority New Zealand Certificate in Science one. Any technical institute able to provide the course would be able to teach it.

University graduates who wish to participate in the training programme should obtain no exemption from the medical technology part of the course. The only exemptions they would obtain would be in the basic science area.

A list of training laboratories would need to be compiled and it would be incumbent on those laboratories to accept students after the two years at technical institutes for work experience. Such laboratories would be mainly the metropolitan and medium sized hospital laboratories.

Students starting the first of their work experience years in the training laboratory could start on the present third year trainees' salary. On completion of their second year in the training laboratory they could obtain the Basic Training Certificate rate.

Technicians would apply for technology training positions in recognised training laboratories. Successful applicants would spend a minimum of 3,500 hours in the discipline. The level of the course would be pitched at the present Part III. On successful completion of the course they would obtain their Diploma in Medical Laboratory Technology and move on to the staff technologists' salary scale.

Training Laboratories and the Teaching of the Diploma Course

The philosophy of this course could be based on the following:

(a) Select students who have demonstrated their ability and are appropriately motivated.

(b) In-service training on a pathology registrar type basis.

(c) Students would largely teach themselves but would be guided by tutorials and some lecturing where appropriate. Their practical training would be similarly guided.

Laboratories would apply to the Medical Technologists' Board for recognition as a training laboratory. All such applications would be examined by a special sub-committee of the Board appointed for this purpose. The sub-committee would make its recommendation to the Board on the basis of agreed minimum standards which the applying laboratory would need to attain. Such recognition could be withdrawn if the minimum standards were not maintained.

University graduates wishing to take a diploma would have to obtain the Basic Training Certificate before proceeding. There would be no credits in the diploma course whatever the majoring subject in the degree.

The examinations at the completion of the diploma course would be theory and oral only.

The present technical assistants' programme could continue.

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The Use of Urinary Oestriol in Monitoring Foetoplacental Function

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Based on a paper read to the South Island Seminar. Timaru, May, 1974.

Introduction

In just over ten years the ability to treat the foetus as a patient and recognise foetal dysfunction has become possible. The estimation of the steroid hormones, the oestrogens, is now a widely accepted and well-published means of assessing foetoplacental well-being. It is intended to show the usefulness of urinary oestrogens in assessing foetoplacental well-being.

Source

During the first few weeks of pregnancy the role of oestrogen production is taken over from the ovaries and adrenal cortex by the foetus and placenta. Both the foetus and placenta then work together as a unique endocrine unit producing oestrogens (Figure 1).

Throughout pregnancy there is a steady rise in the production of all the oestrogens. Oestrone and 17β oestradiol increase one hundred fold from a few micrograms per 24 hours, whereas oestriol increases by approximately one thousand fold[1, 12, 31]. It is because of this extremely high increase during pregnancy that oestriol is generally measured.

Excretion

Oestriol is excreted via the maternal

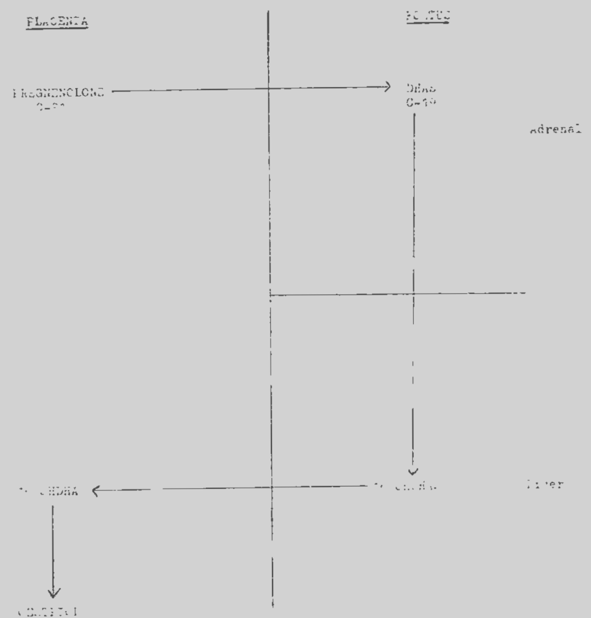

Figure 1.—Simplified diagram of oestriol production from pregnanolone Non-standard abbreviations: DHAS Dehydroepiandrosterone Sulphate. 16OHDHAS —16α Hydroxy Dehydroepiandrosterone Sulphate. 16OHDHA—16α Hydroxy Dehydroepiandrosterone ERRATUM in Diagram. Pregnanolone not Pregnenolone.

kidneys as either oestriol glucuronide and sulphate or as a sulphonated glucuronide. The conjugation takes place in the maternal liver.

Urinary oestriol excretion shows a marked diurnal variation, therefore, it is generally necessary to collect a 24-hour urine. Should this be impossible Salvadori *et al.* [36] have shown that the best correlation with a 24-hour urine oestriol is obtained when the urine is collected between 1300 hours and 1900 hours. This fraction has a correlation co-efficient of 0.907 when compared with the 24-hour oestriol.

The usefulness of the oestriol-creatinine ratio has been confirmed by a number of workers [9, 10, 28, 33, 41] particularly with random urines. Here again Salvadori *et al.* [36] showed that the best correlation co-efficient of the oestriol-creatinine ratios against that for the 24-hour urine was the 1300 hour to 1900 hour urine specimen with a correlation co-efficient of 0.961.

Some workers[21, 26, 37, 40] have shown that the oestriol-creatinine ratio was an insensitive means of monitoring certain high risk pregnancy conditions such as hypertensive complications. Harding and Spence[21] have shown that in some cases of toxaemia with marked involvement of the foetus, the oestriol-creatinine ratio may well be within normal limits. They suggest that when both the 24-hour oestrogen and creatinine concentrations are below normal, a creatinine clearance be determined when the urine collection appears to be a 24-hour urine.

In the author's laboratory both the 24-hour oestriol and creatinine excretion are calculated. An oestriol-creatinine ratio is calculated but is used only to demonstrate trends; the 24-hour oestriol excretion is used as the definitive result. The overall oestriol result is assessed on the basis of a 24-hour urine volume, the oestriol result, the 24-hour creatinine result and the oestriol-creatinine ratio before release from the laboratory. A creatinine clearance is also estimated on each patient when oestriol estimations are first initiated to assess the patient's renal function.

Assay

Numerous methods have appeared in the literature over the last five years. Besides the use of more sophisticated techniques such as Gas Liquid Chromatography (GLC) and column fractionation, the majority of methods have employed either acid or enzyme hydrolysis of the oestriol conjugate, solvent extraction and the Kober chromogen reaction.

Kober[25] in 1931 demonstrated that when oestrogens were added to a mixture of sulphuric acid and phenol, heated, diluted with water and reheated, a pink colour resulted which absorbed light at 514nm. Subsequent work has shown that other phenols and some reducing agents can be substituted for phenol.[1, 2] Hydroquinone is commonly used now instead of phenol.

In 1958 Ittrich[22] demonstrated that the oestrogen/Kober chromogen reactant could be extracted into a paranitrophenol/chloroform mixture and measured fluorometrically. This increased the sensitivity of the method by more than forty times. Ittrich[23, 24] also showed in 1960 that other organic solvents could be used to extract the Kober colour, such as ethanol in acetylene tetrabromide and tetrabromomethane.

In 1968 Brown *et al.*[3] demonstrated the effects of time, temperature and the concentration of sulphuric acid in the Kober reaction.

Lever *et al.* (1973)[27] used the traditional Kober reaction then diluted with chloral hydrate/trichloracetic acid to give an Ittrich fluorescence. This method does not employ an organic solvent extraction and easily lends itself to automation.

The Kober chromogen reaction is influenced by the presence of reducing sugars in urine, and some workers have reported as much as 40 percent quenching with a glucose concentration of 1 to 2 g/dl.[4, 6, 8, 20, 42].

It has also been observed that an inhibitor to the Kober oestrogen reaction is present in some fresh urine specimens which will yield low recoveries of added oestriol. The interference of the inhibitor decreases with the age of the specimen. The structure and nature of the inhibitor. is as vet. unknown[33].

Clinical Significance

Normal Pregnancy. During early pregnancy oestriol levels are extremely variable, however, after approximately 30 weeks gestation urine oestriol correlates extremely well with increasing activity of the foetoplacental unit. The rise in the oestriol concentration compared with the period of gestation is clearly demonstrated (Figure 2). The use of isolated oestriol assays is of limited value and

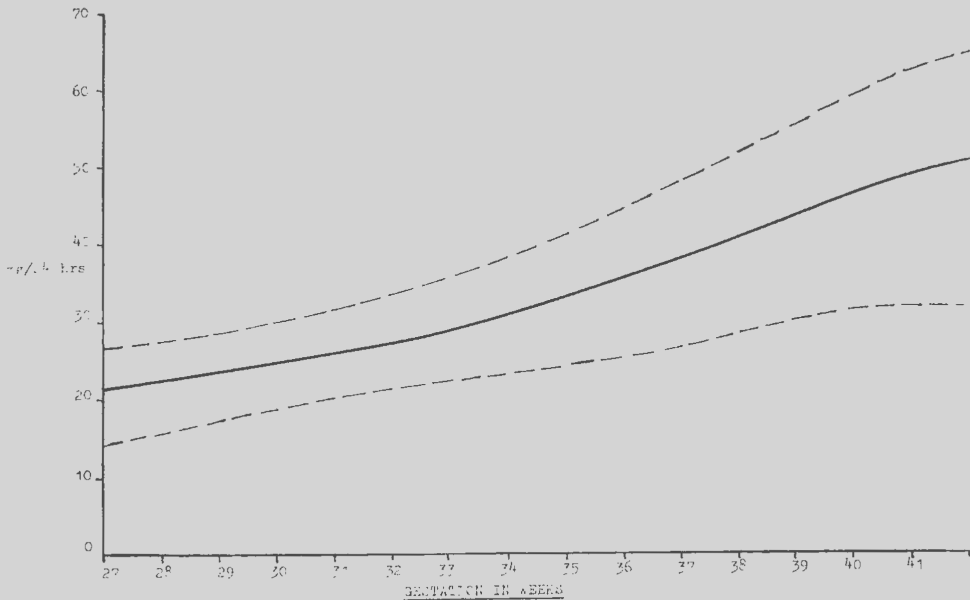

FIGURE [illegible]: EXCRETION OF URINARY OESTRIOL DURING A NORMAL PREGNANCY.

serial estimations are essential to produce an accurate foetoplacental function picture.

Abnormal Pregnancy

Pre-eclampsia. Urine oestriol levels are in the lower level of the normal range. In patients with moderate and severe pre-eclampsia urine oestriol levels are abnormally low for the stage of pregnancy.

Normal pregnant women with a small heart size can show urinary oestriol levels in the same range as pre-eclampsia patients[38].

Threatened Abortion. A downward trend in serial urine oestriols with a sudden drop indicates impending abortion. If abortion is prevented oestriol levels usually rise to the lower part of the normal range.

Placental Insufficiency. This is a poorly defined condition and urine osetriols are usually abnormal. Often there is a failure to show a progressive rise during the course of the pregnancy. Charles *et al.*[5] showed that when pregnanediol/oestriol ratios from five pregnancies with placental insufficiency were compared with a normal control group, the former group had higher ratios during pregnancy and had a marked fall prior to delivery. The authors attributed the changes in the ratios to differences in the relative contributions of the foetus and placenta to the excretion of pregnanediol and oestriol in the maternal urine.

Diabetes Mellitus. During a normal diabetic pregnancy oestriol levels are normal or lower limit of normal. No correlation, to date, has been demonstrated between the severity of diabetes mellitus and urinary oestriol excretion[17, 18]. If oestriols are determined by the Kober chromogen reaction then attention should be paid to the level of reducing sugars present in the urine prior to analysis.

Rhesus Iso-Immunisation. Urine oestriols are within the normal range and there is no correlation with the severity of the disease. A correlation between intrauterine transfusions and plasma oestriol levels has been demonstrated [34].

Foetal Death. Foetal death is often preceded by fluctuating oestriol levels and a sudden drop indicates foetal death [16, 19]. Greene and Touchstone[19] also showed that in patients in whom neonatal death had taken place urinary oestriol levels are generally abnormally low.

Placental Sulphatase Deficiency and Congenital Adrenal Hypoplasia

A small number of cases have been reported where the urinary oestriol levels during the third trimester were very low, in the order of 1 to 3 mg per 24 hours [13, 14]. However, a normal child was produced at

birth. It has been demonstrated that in these cases the placenta was deficient in the enzyme placental sulphatase[14].

The second rare group is where the foetal adrenal is hypoplastic and fails, therefore, to produce oestriol precursor dehydroepiandrosterone sulphate which results in abnormally low oestrogens[35]. In both of these conditions the pregnanediol excretion is normal[13, 14, 35].

Anencephaly. Abnormally low urinary oestriols for the appropriate stage of pregnancy in women with anencephalic foetuses have been reported by a number of workers[7, 15, 30].

Multiple Pregnancies. Urinary oestriols are normally in the upper limit of the normal range or abnormally high in multigravida states. Pregnancies where the foetuses share the same placenta may often be well within the normal range.

Prolonged Pregnancy. Urinary oestriol levels continue to rise in prolonged normal pregnancies. However, other observations generally prove more useful in assessing the length of pregnancy such as ultrasonics, weight and girth measurements.

Child Development after Pregnancies with Low Urinary Oestriol Excretion

Workers[30, 44], doing preliminary follow-up examinations on some children whose mothers had low urinary oestriols and/or pre-eclampsia during the pregnancy, have shown that the longer the low oestriol values persist the more likely the child is to be mentally retarded. In pregnancies where there were precipitately falling oestriol levels, and the foetuses were delivered for that reason, the children developed better than children in the group of pregnancies with continually low oestriol levels.

Discussion

The use of urinary oestriol in assessing the status of the foetus as a patient is well established. Urinary oestriol offers many advantages over the "newer" placental function tests such as human placental lactogen (HPL): (1) it is a well documented system for assessing foetoplacental function; (2) it is a relatively inexpensive and simple assay to perform; (3) in conjunction with other assays such as pregnanediol it can aid in foetal diagnosis. Finally, the possibility of predicting future child development patterns introduces an exciting extension of the now conventional oestriol assay and foetoplacental function assessment.

It must be stressed, however, that the use of single oestriol assays is of little diagnostic value. Serial oestriols are essential to provide an accurate foetoplacental function picture.

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Urine Protein Determination Utilising Eosin-Y in a Continuous Flow System

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Summary

A method for quantitation of protein in urine using eosin-Y is described. Correlation with a turbidimetric urine protein method is acceptable (r = 0.975). The technique is particularly suitable for continuous flow analysis being simple, inexpensive and reproducible.

Introduction

Urine protein is commonly determined by the automated turbidimetric procedure of Brugerie *et al.* (1966)[1]. Methods of this type produce satisfactory results in short runs. However, significant base line changes are caused by the protein-sulpho-salicylic acid complex adsorbed onto the flow cell walls. Blank values are also required necessitating a dual channel system or a re-running of all samples a second time. A review of potential methods to overcome these disadvantages was initiated by Lever (1973)[2] who suggested utilising the quenching effects of protein on the dye eosin-Y as reported by Hiraoaka *et al.* (1963)[3] This paper describes a study of the use of eosin-Y for the determination of protein in urine by fluorometric continuous flow analysis.

Materials and Methods

(a) Reagents for eosin-Y method.
1. L (+) Tartaric acid (2M)
2. Sodium hydroxide (5M)
3. Eosin-Y (yellowish) (0.01M) either Harleco, item 200, or George T. Gurr Ltd.
4. Pooled bovine serum (71 g l^{-1}) acquired from the local abattoir
5. Working eosin reagent. Eosin-Y (30μM), tartaric acid (0.3M), sodium hydroxide (0.2M), pooled bovine serum (0.213 g l^{-1}).
   Adjust pH to 3.2±0.1. The reagent is ready for use after one hour (Figure 1).

(b) Reagents for turbidimetric method.
1. Sulpho-salicylic acid (0.2M) with sodium sulphate (0.07M).
2. Sodium chloride (1.7M).
3. Blank reagent—distilled water without added surfactant.

(c) Standards

Pooled, non-haemolysed, clear human serum was analysed to ascertain its protein concentration by the autoanalyser serum biuret method (Technicon N method file N-14b). The serum biuret procedure was standardised with Boehringer Mannheim Preciset protein standards. Dilutions of pooled serum were made over a range of 0.1-7.5 g l^{-1} using a diluent of 0.15M sodium chloride containing 0.015M sodium azide as preservative.

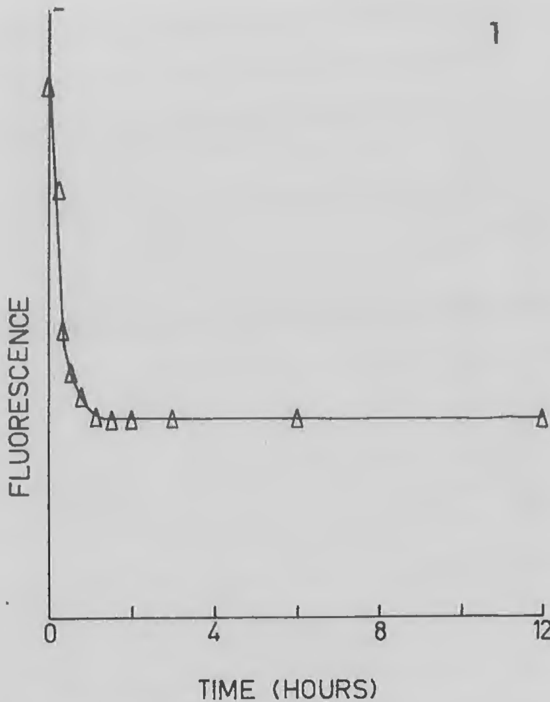

Figure 1.—Initial fluorescence decrease exhibited by working eosin reagent.

(d) Equipment

Basic unit technicon autoanalyser modules were used. The colorimeter had a specially modified flowcell and a short mixing coil immediately prior to the debubbler to ensure the precipitate remained in suspension. The fluorometer for the automated eosin method was a Perkin Elmer model 203 fluorescence spectrophotometer fitted with Xenon arc source lamp and Aminco Bowman pyrex flow cell with integral debubbler (part no. 4-7413). Results were recorded on a Honeywell Brown Electronik 10 millivolt full scale recorder fitted with a 50 cm per hour chart drive. Manual fluorescence measurements were made using an Aminco Bowman spectrophotometer fitted with a Xenon arc lamp and R136 photomultiplier. Mirrors and 1 mm slits were placed in the cell housing. Fluorescence data were not corrected for instrument response.

(e) Specimens

Both procedures utilised aliquots of centrifuged 24 hour urine collected mainly from women in the third trimester of pregnancy.

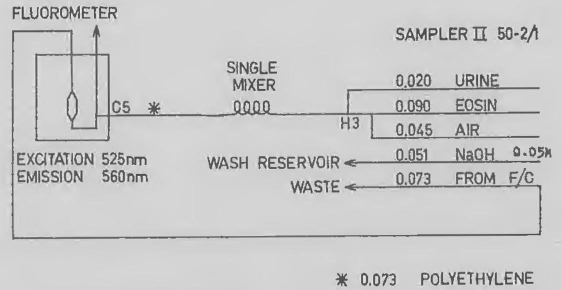

Figure 2.—Flow diagram of the continuous flow eosin protein procedure. Polyethylene transmission tubing is used wherever possible. Pump tube internal diameters given in inches.

(f) Methods

1. Automated eosin procedure.

This is illustrated by the flow diagram in Figure 2. Sample is added to the eosin reagent in the ratio of 1:18, mixed by passing through a 14 turn 2.4 mm I.D. mixing coil, pumped through polyethylene tubing to the fluorometer where fluorescence is measured at excitation and emission wavelengths of 525 nm and 560 nm respectively.

2. Automated turbidimetric procedure.

The method used is that of Brugerie *et al.* with two small modifications. The sodium chloride concentration is increased from 1.5M to 1.7M and the sample line was decreased from 0.040 mm I.D. to 0.035 mm I.D. The urine sample is diluted with 1.7M sodium chloride, mixed in a 14 turn coil, sulpho-salicylic acid reagent added and the mixture passed through two 28 turn 2.4 mm I.D. mixing coils. The percentage transmission is then measured at 625 nm. Blanks are determined by substituting distilled water for all reagents and re-running all specimens. The blank is then subtracted from the test to give the final result.

Results

The effect of adding increasing amounts of protein to the eosin working reagent is shown in Figure 3. A solution of protein was added to the Eosin working solution continuously and stirred with a magnetic stirrer. The mixture was aspirated directly into the Autoanalyser manifold and the optimum protein concentration calculated on a time basis. The natural fluorescence is first quenched, then, at higher protein concentrations, the fluorescence is enhanced. Sufficient protein is

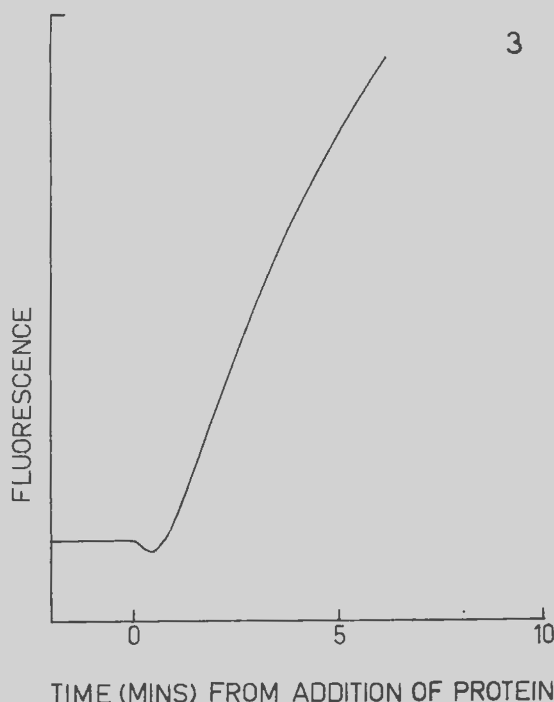

Figure 3.—Change in fluorescence shown by continuous addition of bovine protein 71 g l^{-1} at 0.16 ml $min.^{-1}$ to working eosin reagent decreasing constantly at a rate of 2.5ml per min.

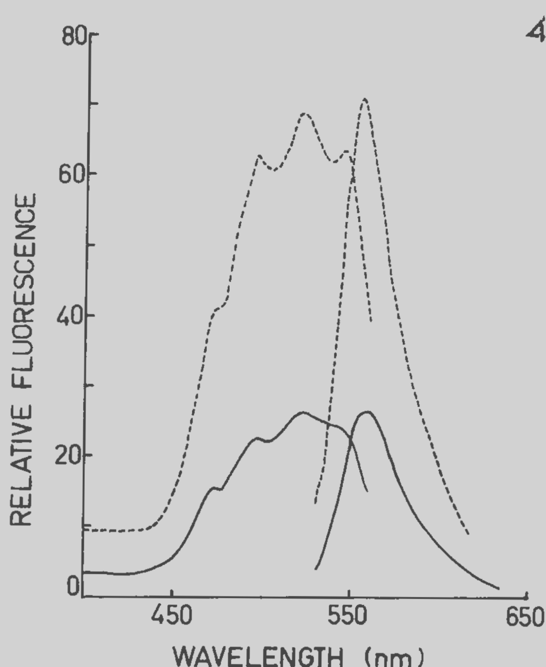

Figure 4.—Excitation and emission spectra of eosin-Y. Solid line shows spectra for autoanalyser working reagent. Dotted line shows increase in fluorescence with added protein.

added to the reagent to utilise the portion of the curve where fluorescence is directly proportional to protein concentration. In the method described sufficient protein was added to produce a fluorescence slightly above the base line level. Sensitivity of the procedure can be altered by adding more or less protein to the reagent.

In Figure 4 the working reagent's characteristic fluorescence is shown by the unbroken line, the broken line represents the enhancement of fluorescence in the presence of protein. Excitation and emission maxima are at 525 nm and 560 nm respectively.

The responses of both methods to different protein fractions (Table 1) show that the best correlation is with human albumin. Table 2 shows that there is no significant response by either method to some substances that occur normally in urine even at four times the upper limit of normal concentration.

Evaluation of Procedure

Recovery ranged between 94-104 percent when pooled human serum (which was used as the standard) was added to a urine initially containing less than 0.3 g l^{-1} protein. Reproducibility as measured by 20 determinations on one sample gave a coefficient of variation of 1.6 percent at a level of 1.2 g l^{-1} protein. The coefficient of variation for the turbidimetric method for the same series was 4 percent.

A study of 24 hour urines mainly from women at various stages of pregnancy showed that 2 percent of the total had more than 0.3

Table 1

Protein fraction 1 gl^{-1}	Relative response against human serum standards	
	Fluorometric	Turbidimetric
Albumin	108	100
Alpha *	10	40
Beta *	5	0
Alpha and Beta	85	75
Gamma	15	75
Glycoprotein	20	5

*Solubility problem was experienced; only method interrelationships are shown.

Table I.—Response to various protein fractions dissolved in physiologically normal saline.

Table II

Substance	Apparent Protein gl^{-1} Fluorometric	Turbidimetric
Potassium	0	–0
Uric acid	0	0
Glycine	0	0.25
Calcium	0	0.40
Sodium	0	0
Creatinine	5	0
Magnesium	0	0
Urea	0	0
Phosphate	0	0

Table II.—Apparent response to a selection of urine components each dissolved in pooled normal urine not containing protein. The concentration of each component approximates four times upper limit normal concentration.

g l^{-1} protein. A correlation coefficient between the eosin-Y and turbidimetric methods on these elevated samples was 0.975, n = 104. The mean for the turbidimetric method was 1.57 g l^{-1} and 1.62 g l^{-1} for the fluorometric method.

In a series of 208 urines reported as containing less than 0.3 g l^{-1} protein, 96 estimated by the turbidimetric method had a significant protein value before the blank was determined, whereas in the fluorometric method all 208 showed less than 0.3 g l^{-1} protein. Comparison of results before and after centrifugation showed that for the eosin procedure, no significant variation occurred (n = 30). Mean before centrifugation, 1.24 g l^{-1} protein after centrifugation, 1.18 g l^{-1} protein, thus valuable savings in time can be made by using the fluorometric method.

Experiments to ascertain the effect of temperature on the fluorescence of both the eosin-working reagent and protein-working reagent complexes showed that, from 20°C to 60°C, the fluorescence of the working reagent decreased by an equivalent protein concentration of 0.0005 g l^{-1} $°C^{-1}$. Reagent plus albumin showed an increase of the same magnitude while reagent plus gamma globulin showed an apparent protein increase of 0.0003 g l^{-1} $°C^{-1}$.

Surfactants, including Brij -35, triton x-100, triton x-405, FC-134, ARW-7 and sodium dodecyl sulphate, were tested to check whether the different specificity for albumin and gamma globulin could be changed. No effect was observed. Concentrations of surfactant up to 5 percent W/V cause an increase in natural fluorescence of the reagent with no added sensitivity to protein. At concentrations above 5 percent W/V fluorescence response diminishes rapidly.

Fluorescence response is directly proportional to protein concentration (Figure 5A). A copy of the autoanalyser standard curve is shown in Figure 5B. Percentage interaction between 0.5 g l^{-1} and 7.5 l^{-1} protein standards was 1.6 percent and percentage steady state (5.0 g l^{-1} protein) was 94 percent.

Discussion

While the automated turbidimetric procedure of Brugerie *et al.*[1] is capable of processing large numbers of specimens it requires blank determinations and suffers from base line instability. In the method described neither of these problems arise.

Approximately 98 percent of all urines analysed gave protein results of less than 0.3 g l^{-1}, and most of the remaining 2 percent greater than 0.3 g l^{-1} were essentially due to albumin excretion[4]. Thus, human albumin standardisation has been used as an alternative to pooled human serum. With either method of standardisation it must be recognised that urine containing proteins other than albumin will show different apparent protein concentrations because both methods (Table I) have different specificities for each protein fraction. Consequently neither method produces an absolute value of protein concentration.

An important aspect of the working eosin reagent is that stabilisation of the natural fluorescence takes approximately one hour. Stability is retained for up to five days at room temperature when stored in a clear glass container. Stability is limited by the growth of micro-organisms.

Fluorometric methods are often temperature sensitive. However, for the eosin procedure a change in temperature of 5°C caused an average apparent increase in protein concentration of 0.0004 g l^{-1} which is insignificant compared to protein levels found in urine.

As noted earlier the presence of high concentrations of surfactants causes a reduction in fluorescence response. However, it was found that the bovine serum present in the reagent functions as a natural surfactant and consequently satisfactory bubble patterns are produced, without additional surfactant. Polyvinyl pyrollidine will also produce good bubble

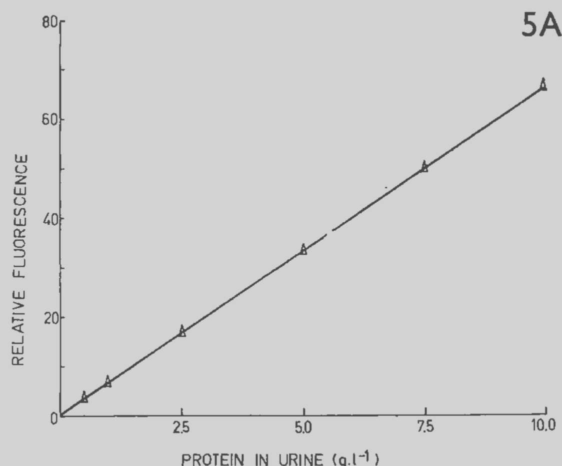

Figure 5A.—Response of automated eosin-Y procedure to different protein concentrations.

patterns. The use of polyethylene tubing where possible improves both lag and exponential factors as predicted by Walker *el al*[5]. Polyethylene tubing adsorbed eosin rather less than glass or tygon tubing. Any slow build-up of eosin may be effectively removed by a five minute wash with a solution of 0.5 percent W/V Pyroneg (Diversey Wallace Ltd.).

Limitations of the method in its present form include a quenching effect caused by large amounts of bilirubin glucuronide in urine. Erroneous protein results will be caused by the addition of hydrochloric acid to urines; in this case results will be falsely elevated. Conversely, strongly alkaline urines (those many days old and bacterially contaminated) will cause falsely low protein results. Both problems are overcome by simple precautions such as using reasonably fresh, properly stored urines, without added acid preservatives. If acid urines must be used, increasing the buffer concentration of the reagent will suffice provided that the protein has not already been destroyed.

The increased demand for the measurement of urine oestriol, creatinine and protein in pregnant women stimulated an investigation of methods to increase the through-put of work. The principal limiting factors were the requirement to run protein blank determinations and fully committed equipment. The use of the automated eosin-Y procedure overcomes these problems and also allows faster sampling rates than those presently used.

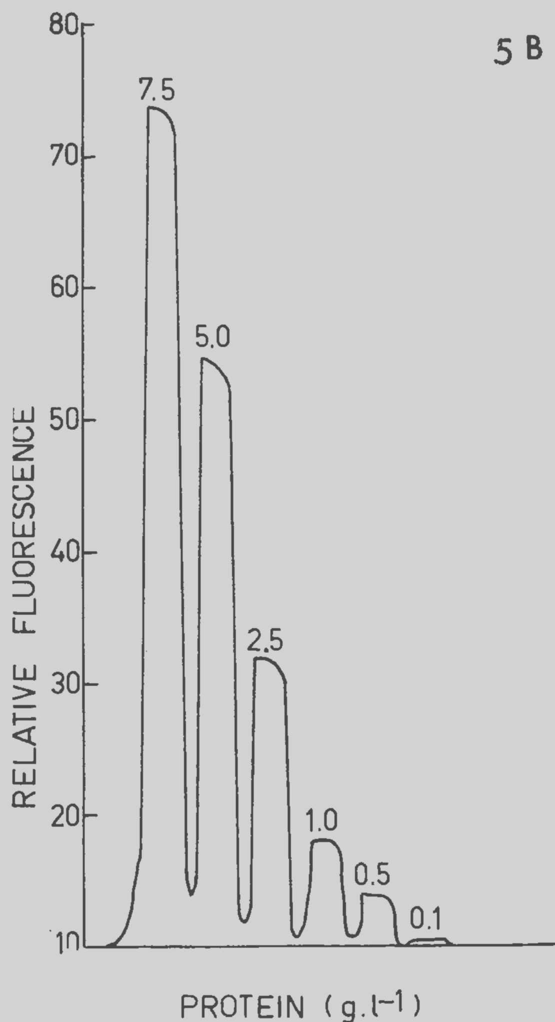

Figure 5B.—Reproduction of autoanalyser recorder tracing for fluorometric protein calibration curve.

Acknowledgments

The author is grateful for the assistance of the technical staff and other past and present members of the Laboratory, Green Lane Hospital.

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Immunological Tests in the Diagnosis of Cancer

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In the last thirty years there has been a vast amount of experimental work studying the immunological features of cancer. Although most of this work has involved studies on experimental tumours in animals particularly in mice, more recent work in man has suggested that most or all these observations are valid in human cancer. A number of important facts have already been established.

1. Tumour-associated Antigens (TAA)

Ludwig Gross, and later Prehn and Main, immunised mice with tumours from syngeneic (identical) mice and found that the immunised mice no longer accepted tumour grafts from their twins. This suggested the presence of foreign antigens on the surface of the tumours, for tissues other than the tumours were not rejected after grafting. These antigens are now referred to as tumour-associated antigens and are specific for the tumour and are not found on normal cells of the same tissue from which the tumour arose. A number of different types of TAA have been recognised (Klein, 1968)[8]. Some chemicals, termed chemical carcinogens such as methyl cholanthrene, will induce tumours in a high proportion of animals into which they are injected. TAA in this instance are specific for the tumour and not for the chemical. That is, if a hundred tumours were induced by methyl cholanthrene a hundred different types of TAA would be found. Some viruses (oncogenic viruses) will induce tumours in certain strains of animal, but here the TAA cross react in that they are specific for the virus and will occur on all the tumours produced by the one virus. There is still no unequivocal evidence that any human tumours are induced by viruses but it seems most likely that many are.

On some human tumours, antigens of embryonic origin appear. These antigens which are present during foetal life are no longer expressed in adults but will appear sometimes in large amounts on the surface of some tumours. Finally, there are some tumours on which no detectable TAA have been found. This may be because they express no such antigens or because the methods of detection are still too insensitive.

Neuroblastomas, melanomata, and bladder carcinoma in man have quite strong TAA and they have also been found on many other tumours including some leukaemias (Harris, (1973)[6].

2. Immunological Surveillance

When a patient's immune system is depressed for a long time there is an increased incidence of tumours. This occurs in children with some forms of severe cogenital immunodeficiency disease and in some patients being treated with high doses of immunosuppressive drugs for a long time, as with patients after renal transplants. However, the incidence of tumours in both these situations is still low enough to suggest that we are far from understanding the full mechanism for recognition and disposal of malignant cells. Tumours may well develop when the immune system is too slow to detect a malignant change. The hypothesis that the immune system functions as a protective agent seeking out and destroying potentially malignant cells is termed immunological surveillance (Burnet 1970)[1]. Probably tumours with strong TAA are most readily stopped by the surveillance system. Immunity to many antigens including tumours is very dependent upon genetics. The capacity to respond to many antigens and probably to TAA is inherited and is closely related to the inheritance of histocompatibility antigens. Thus some people may be more susceptible than others to tumours which arise with particular TAA.

3. Escape from Surveillance

Tumours may develop by sneaking through or sneaking past the immune defences. An

experiment by Old where low, middle, or high numbers of tumour cells, were inoculated into a mouse strongly suggested this. The results of his experiments suggested that a moderate inoculum of tumour cells might well be recognised for its foreign nature and be promptly rejected, whereas a very small inoculum of tumour cells might be ignored if the stimulus of its TAA was too small. When the tumour had grown sufficiently large to be recognised it might already be too large to be rejected. Some tumours may develop immune resistance against the defence mechanisms while other tumours may have very low levels of TAA or may arise in sites such as the breast where large amounts of antigen are needed to provide an immune response. In other sites such as the skin, very small amounts of foreign antigen, such as TAA, will provoke a strong immune response (Klein, 1973)[9].

4. Host Inmmune Responses

An immune response appears to be activated against most cancers. This involves the two major components of the immune response, humoral immunity with production of antibody and cell-mediated immunity which depends for its effect upon a direct cell to cell contact between T lymphocytes, or T lymphocytes and macrophages, and the target cancer cell. Antibody may be cytotoxic and with the aid of complement destroys target cells, or antibody may act by sensitising tumour cells and activating K cells of the lymphocyte series to attack the tumour cells. The cell-mediated response is really the standard acute rejection mechanism which is involved in rejection of skin grafts or kidney grafts. Hellstrom and Hellstrom have identified serum components termed "blocking factors" which inhibit this effective cell-mediated response. These blocking factors now appear to be antigen-antibody complexes where the antigen is TAA. The continued growth of a tumour may well be related to the presence of these blocking factors as their spontaneous disappearance has been seen to precede the spontaneous regression of a melanoma in man (Hellstrom and Hellstrom, 1971)[7].

5. Generalised Depression of Immunity

With almost all patients with cancer with some tests (see below) some defective lymphocyte function is seen. Although it is not established whether this depression of immunity precedes the cancer it is more likely to be a result of the development of tumour. But this depressed immunity encourages continued growth of the cancer.

Laboratory tests in cancer:

(a) Identification of lymphocyte sub populations

T and B lymphocytes can be identified in the peripheral blood by using markers for these cells. B lymphocytes carry surface immunoglobulin, many carry Fc receptors and complement receptors, while T lymphocytes are demonstrated by their binding of sheep red blood cells in vitro (Wilson, 1974)[11]. In chronic lymphocytic leukaemia (CLL) almost all patients have a monoclonal expansion of B lymphocytes. The surface immunoglobulin on these cells is almost exclusively one heavy and one light chain class. Occasional patients have been described with T cell lymphocytic leukaemia. The pattern and the density of labelling may have prognostic and diagnostic significance in patients with CLL and lymphosarcoma. One communication has suggested that a decreasing density of surface immunoglobulin in lymphosarcoma indicates a poor prognosis. These labelling techniques are of value in demonstrating that a lymphocytosis is clearly due to CLL or to lymphosarcoma. CLL lymphocytes are exquisitely sensitive to Colchicine in vitro, and this may also be used as a marker for their presence.

(b) PHA responsiveness of T lymphocytes

A general index of cell-mediated immunity is the capacity of T lymphocytes to be stimulated into cell cycle on exposure to phaetohaemagglutinin (PHA). This response is readily determined using peripheral blood lymphocytes cultured in microtitre trays. The PHA response is depressed in most patients with Hodgkin's disease and the degree of depression parallels the extent of the malignant involvement (Levy and Kaplin, 1974)[10]. Whereas most workers have cultured lymphocytes for three days it appears that a 20 — 24 hour culture, estimating protein synthesis instead of DNA synthesis as the end point, may be more sensitive in detecting abnormalities. This assay has proved to be positive in almost all patients with nasopharyngeal carcinoma (Chan, 1974, personal communication) and in other tumours.

(c) Carcinoembryonic antigen (CEA)

This antigen was first described by Gold

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Maltaner Antigen (DG)	VD 01	5ml
PPR Antigen (DG)	VD 04	10ml
	VD 21	100ml
Reiter Antigen	VD 19	1ml
	VD 20	5ml
VDRL Antigen (DG)	VD 02	5ml
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	VD 25	50ml
Wassermann Antigen (DG)	VD 06	10ml
Haemagglutination Test		
TPHA Kit	VD 29	100 tests
Fluorescent Treponemal Antibody Absorption (FTA-ABS) Test		
Treponemal Antigen dried	VD 28	5 × 1ml
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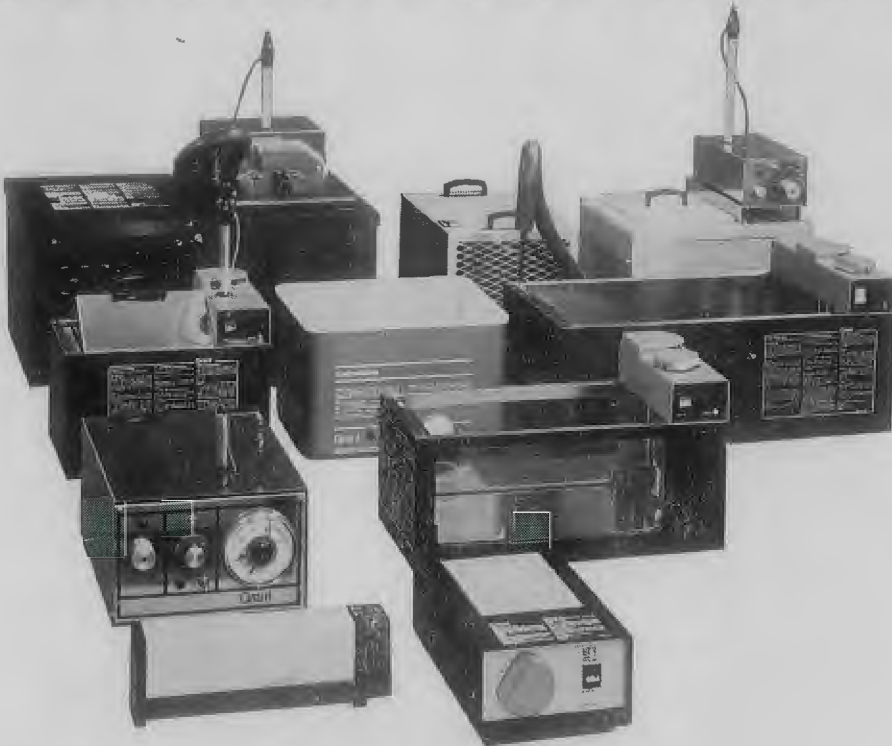

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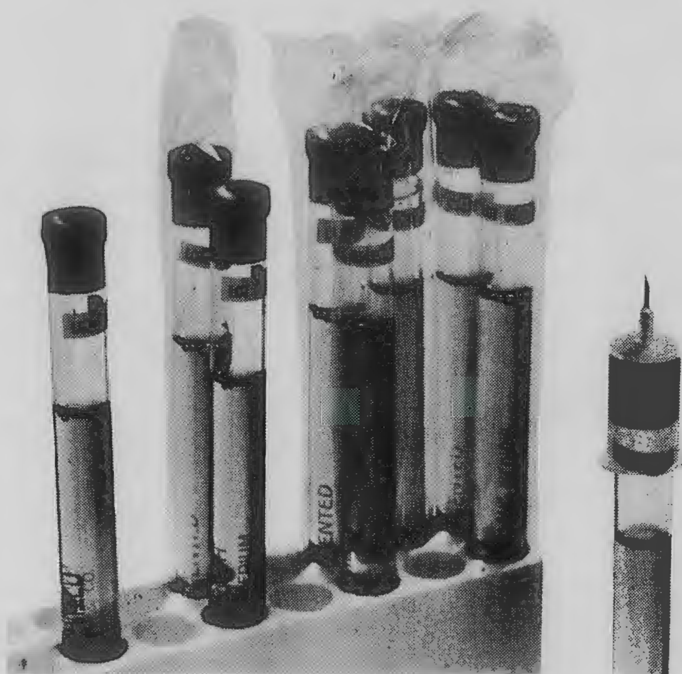

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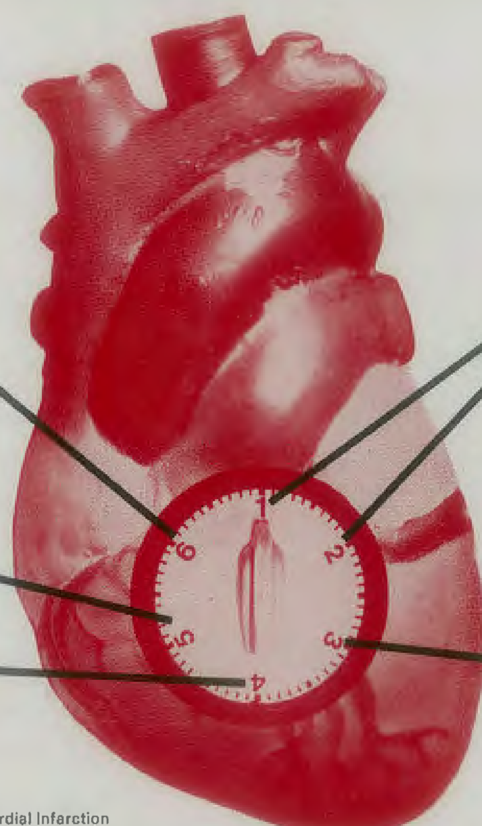

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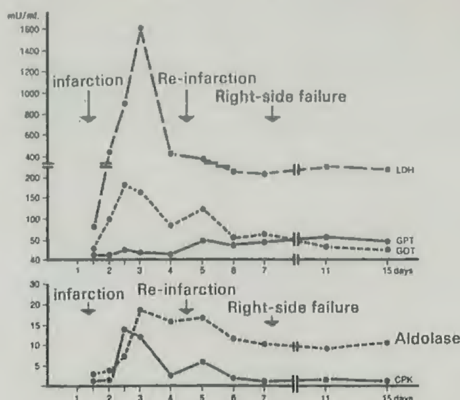

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in Montreal in 1965 (Gold *et al.,* 1965)[4]. It was extracted from carcinoma of the colon and also from foetal gut epithelium. More recently it was extracted from liver metastases. Gold's original observations suggested that this antigen was specific for tumours of the colon and the antigen was found in the serum of those patients. Since then a number of false positives have been found but the assay still has some clinical significance. A radioimmunoassay for CEA has been developed using antibody raised in rabbits, or goats, which is then absorbed with normal tissues and plasma. CEA is extracted from serum or plasma with perchloric acid, the supernatant is dialysed and then anti-CEA antibody is added followed by I^{125} labelled CEA and the bound CEA is precipitated with zirconyl phosphate gel. A commercial kit for this is now available and using this Hansen *et al* (1974)[5] have recently reported the result of 35,000 tests. The normal levels were less than 2.5ng/ml and 73 percent of patients with gastrointestinal tract carcinoma had levels above 2.5. 91 percent of patients with pulmonary carcinoma have raised levels, and in 50 percent of patients with these two tumour types, the levels were about 5.0ng/ml. However, there are a large number of non-malignant conditions where the titres were raised although in general only mildly; emphysema, alcoholism, cirrhosis, colitis, gastric ulcer, bronchitis and in cigarette smokers. The assay appears to be of most value when the titres are high though this usually means an advanced cancer, and it can be used to monitor patients following surgery. Rising titres after operation indicate the presence of metastases.

(d) Macrophage electrophoretic mobility test (MEM)

This test was devised by Field and Caspari (1970)[3], and depends upon the capacity of some proteins to release a factor from lymphocytes of patients with cancer which then slows the migration of macrophages in an electrophoretic field. The proteins used for this are encephalitogenic factor (EF) or cancer basic protein (CBP). Almost all patients with cancer, or who have had cancer, give a positive result on this test and the number of false positives is small. However, some patients with neurological disease, sarcoidosis, SLE and asthma may give false positives. It cannot be used as a screening test because the test is slow and very cumbersome.

(e) Antibody versus lymphoblastic leukaemic cells

Greaves at University College in London has prepared an antibody in rabbits against acute lymphoblastic leukaemic cells (ALL). The serum was repeatedly absorbed to give specificity. The resultant antibody tagged with fluorescein is able to demonstrate cells bearing antigen apparently related to the leukaemia in the bone marrow and peripheral blood of children with ALL. Less than 0.02 percent of normal bone marrow cells label with this antibody but in children with ALL, even in remission, up to 50 percent of their bone marrow cells labelled. Some of these cells had the appearance of blast cells but others have the appearance of large lymphocytes. This technique and related approaches are likely to improve therapy by detecting relapses at a very much earlier stage.

(f) The biophysical difference between lymphocytes from healthy donors and patients with malignant disease.

A new assay has been devised by Cercek *et al.* (1974)[2] which depends upon the structuredness of cytoplasmic matrix of lymphocytes, and changes in this matrix, induced by PHA or cancer basic protein, which are then studied using fluorescence polarisation. The assay is relatively simple, and significant differences have been shown between healthy patients with malignant tumours. This test may become the single most effective test in the early diagnosis of cancer. However the results are still preliminary and it will require another two to three years to establish its full significance.

It is likely that in the next few years a number of immunological tests, both for the detection and the follow-up of patients with cancer, will be devised. Some of these will depend upon lymphocyte function, while others will depend upon the identification of tumour specific antigens present in the serum of the patients. These tests are likely to add greatly to the effectiveness of the diagnosis and the management of cancer.

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An Example of an Antibody to Altered Albumin

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Received for publication, October 1974

Summary

This paper describes an example of an antibody active against altered albumin which was found in the serum of a 52-year-old male.

Details of thermal amplitude and critical albumin concentration are given as are results of tests with various brands of albumin.

Details

In September 1974 Mr A. L. P., aged 52 years, was admitted to Iona Private Hospital in New Plymouth for prostatectomy. He had no history of having had transfusions or blood products.

A two unit crossmatch was requested, the patient grouped as O positive (R_1r) and two units of O positive donor blood were selected and a crossmatch set up using our routine 20 minute spin Lows-papain technique, one hour albumin/Coombs technique, and one hour room temperature-saline technique, in all cases a 3-5 percent suspension of washed cells were used.

Macroscopic reading over a concave mirror (after centrifugation) of the albumin crossmatch showed strong visual agglutination with both units, and the patient's own cells, run as an auto-control. The crossmatch was repeated using the original two units plus eight further units with a saline Coombs technique and an albumin addition technique in addition to the albumin/Coombs crossmatch. All units including the original two were found to be compatible by all techniques, except for the albumin part of the albumin/Coombs crossmatch, where strong visual agglutination was evident with all units tested, including the auto-control. The albumin addition technique, where albumin is added to saline suspended cells and serum after one hour's incubation and read ten minutes later microscopically did not detect any incompatibility, neither did the saline Coombs crossmatch. When the incubation period was extended to 30 minutes after the addition of albumin, agglutination once again appeared in the albumin addition method. One unit of packed cell was transfused post-operatively without any untoward effect; and the antibody was still present two days post-transfusion.

The original example of this antibody was described by Weiner *et al.*[4] who found optimum reactions at 37°C, Powell and Rees and other workers showed reactions to be equally strong at 4°, 20° and 37°C with titres in excess of 1:8. Our antibody appears to conform to the second set of reactions but only had a titre of 1:2. However, as Powell and Rees found[3], the titre increased when left standing overnight at 4°C to a titre of 1:8. Bird and Wingham[1] have reported an anti I which acts preferentially in albumin and by testing against cord cells it was shown that Mr A. L. P.'s serum reacted equally as strong as with adult cells showing it not to have I specificity. The serum was tested in Dunedin by courtesy Mr D. S. Ford on the Low-Ionic Strength Analyser and showed there to be no iso-antibody present in addition to the albumin antibody.

Various brands of bovine albumin were diluted in normal saline and tested against the cells and serum of Mr A. L. P. Commercial bovine albumin examined with this serum were from Schering Diagnostics, Armour Pharmaceutical Co. Ltd., and Povite Product (Hol-

land) Ltd., supplied in 30 percent concentrations and the 22 percent concentration supplied by Gamma Biologicals. Concentrations of 30 percent, 20 percent, 15 percent, 10 percent and 5 percent were used in all cases except when Gamma Biologicals' 22 percent bovine albumin was being investigated and here concentrations of 22 percent, 16½ percent, 11 percent and 5½ percent were used. At the same time a sample of human albumin (Commonwealth Serum Laboratories) was tested at a concentration of 25 percent. These dilutions were tested at 37°C, room temperature, 15°C and 4°C, for one hour, centrifuged, and examined macroscopically and microscopically for agglutination of red cells.

The serum of Mr A. L. P. agglutinated his own red cells when 10 percent or higher concentrations of albumin were used in each case except when Gamma Biologicals product was concerned. Here no agglutination occurred even at 22 percent even though other brands showed strong visual agglutination —including the sample of human albumin.

Golde, McGinnis, and Holland[2] in a very informative paper showed that the phenomenon was due to the adsorption on to red cells of an antigen/antibody complex formed by the interaction of altered albumin and the corresponding antibody, a gamma globulin. The chemicals responsible for altering the albumin are acetyl-tryptophanate or caprylate which are fatty acids added to albumin as stabilisers to prevent denaturation when heating during the manufacturing process.

It would appear from these findings that a phenomenon which can be a real problem when crossmatching (it may be masking another antibody) can be overcome either by using a saline Coombs and an albumin addition technique, or by keeping some "unaltered bovine albumin" in stock. However the rarity of this phenomenon would make the first solution a time and serum consuming method while the second solution would require that additional brands and batches of 30 percent bovine albumin be tested with an appropriate serum to find one which does not react. This rarity would preclude any change in technique or brand of bovine albumin but it is essential that the staff employed are made aware of the existence of such an antibody and have alternative methods to turn to to find compatible blood.

Golde *et al.*[2] state that infusion with human albumin to patients with this factor would appear to be contra-indicated and it would be prudent to transfuse plasma to these people.

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A Rapid and Simple Method of Serum Gentamicin Assay

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Received for publication, September 1974

Summary

A rapid and simple method of Gentamicin Assay is presented, using an agar diffusion technique with DST Agar (pH 7.9), and a resistant member of the Klebsiella group as the assay organism.

Introduction

The need arose for a simple method for gentamicin assay in a laboratory required to perform the test only occasionally. As the clinical value of the assay is greater when results are available on the day of the test, a

rapid method was sought. Preliminary work using Diagnostic Sensitivity Test Agar Base (Oxoid), Neomycin Assay Agar (Difco) and Streptomycin Assay Agar (BBL) showed that DST Agar adjusted to pH 7.9 gave the most satisfactory results. A *Bacillus cereus* spore suspension (Difco) and a resistant member of the Klebsiella group NCTC 10896, were the assay organisms used, and the Klebsiella was found most suitable for a rapid test.

Materials

Assay Organism: The Klebsiella culture used was resistant to most antibiotics except the aminoglycosides. It was maintained in freeze-dried ampoules, a new ampoule being opened if any change in sensitivity pattern was observed on weekly sub-cultures. A perfectly level bench was required and this was achieved by mounting a 12in x 12in plate glass sheet on plywood, and attaching levelling screws to each corner. Spirit levels were set in two adjacent sides of the platform.

Medium: DST Agar adjusted to pH 7.9 with N/1 sodium hydroxide before autoclaving. This was stored at 40°C in 100ml quantities.

Gentamicin Standards: An accurately prepared 1 percent aqueous solution was generously donated by Essex Laboratories, and aliquots were stored in the deep freeze. Standards were prepared to contain 10, 5, 2.5, 1.25 μg/ml. An extra standard was prepared to act as an unknown control (8 μg/ml). The diluent was pooled antibiotic-free human serum. It was convenient to store aliquots of the prepared standards in the deep freeze, together with serum from the same source for use in diluting the patients' samples.

Beta-Lactamase: 10ml sterile distilled water was added to 1 vial penicillin amido-Lactamhydrolase E.C. 3.5.26, from *B. cereus* 569/H9 (Whatman Biochemicals). The enzyme neutralised the penicillins and cephalothin but not high levels of cephalexin.

Method

1. A 2mm loopful of Klebsiella culture was added to 10ml Trypticase soy broth, and incubated two hours in a 37°C incubator.
2. 9ml of melted, cooled DST agar (pH 7.9) was pipetted into Petri dishes on the level bench and allowed to set, and the surface to dry.
3. A Pasteur pipette was used to flood the DST plates with the two hour broth culture. All excess fluid was removed and the plates allowed to stand at room temperature with the lids off until the surface was quite dry (30-60 minutes).
4. Patient's serum. A blood sample was collected immediately before a gentamicin dose, and one hour after the dose, the results thus indicating the residual level and the peak level reached. The serum was assayed undiluted, diluted 1 in 2 and 1 in 4, the diluent being pooled human serum as used for the standards. If the patient had received any penicillins or cephalosporins up to 48 hours before the test (or up to one week in renal failure patients) the serum and the dilutions were treated with Beta-lactamase, and the assay performed on both treated and untreated samples. (0.1ml Beta-lactamase was added to 0.5ml of serum or diluted serum and the final result multiplied by 6/5.)
5. With a number 5 cork borer attached to a very gentle suction pump, four wells were cut in each agar plate approximately 2cm from the edge and 3cm from each other. Three wells for each standard and sample were labelled in such a way that the wells for any one sample were on three separate plates.
6. Using a new Pasteur pipette for each sample and standard, the wells were filled just to the surface. Do not allow to overflow. The plates were incubated 4-5½ hours at 37°C until zones could be easily read.
7. Dividers were used to accurately measure the diameters of the zones, and the mean diameters for each sample were found. Using semi-logarithmic paper the mean zone diameters of inhibition of the four standards were plotted against the antibiotic concentrations and the four points joined by straight lines. The concentration of the unknown sample could then be read by interpolation from the graph. The dilution that gave a reading between 1.25 and 8μg/ml was most suitable.

Discussion

It will be noted that the procedure requires the full working day to complete and thus the co-operation of clinical staff is needed. The following modifications can successfully be used:

1. When it is known beforehand that an assay is required, the Klebsiella broth culture may be prepared at 2.30 p.m. on the day be-

fore, and incubated for 18 hours at 37°C. For use the culture is accurately diluted 1 in 5 in Trypticase broth.

2. When samples are received in the afternoon the method may be modified by preparing 12ml plates instead of 9ml, flooding with the two hour broth culture, and incubating the assay for 16 hours at 37°C.

Assay of kanamycin in serum by the above methods was not as satisfactory. However kanamycin results were satisfactory when Streptomycin Assay agar was used (at pH7.9), the Klebsiella culture prepared as above and incubating at 37°C for 5-6 hours or 30°C overnight.

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Communication

A Case of Leprosy

Mrs R., a thirty-four-year-old Fijian Indian woman, was admitted to Nelson Hospital on 4.7.74. She had come to live in New Zealand five months previously from Fiji, following her marriage to a New Zealander.

A known diabetic she presented with pyrexia 38 degrees C, cellulitis of the right lower leg, multiple skin ulcerations and superficial burns to the right leg; the latter being caused by sitting too close to a radiator.

Her skin showed multiple old scars and hyperpigmented areas and she had bilateral enlargement of the inguinal lymph nodes.

Clinically there was evidence of sensory loss in both feet extending up the lower legs. There was also evidence of bilateral loss of the deep reflexes of both legs.

The house surgeon who admitted her summarised his admission notes with the following statement that "the overall clinical picture suggested more than a simple diabetic neuropathy and he queried a diagnosis of leprosy, on account of her background and neurological involvement". The patient and her husband at this time flatly denied the possibility.

On 5.7.74., therefore, the case was brought to the attention of the laboratory as a possible case of leprosy.

A skin biopsy from the right lower leg was taken and this showed histological evidence of multiple granulomatous lesions with involvement of peripheral nerves and was strongly suggestive of leprosy. Ziehl Neelsen stains confirmed the presence of numerous acid fast bacilli, resembling *Mycobacterium leprae*, and the combination of these findings was consistent with this being a case of "active" leprosy, of either dimorphous or lepromatous type.

As a result the patient was informed of these findings and she then admitted that she had a history of leprosy dating back to the age of eight and that she had in fact spent more than five years in a leprosy hospital in Fiji.

At this stage it was decided to prepare a series of skin smears from various body sites and where possible to perform a Ridley count, to give a better appraisal of the bacteriological state of the disease.

Ridley Count

	Right	*Left*
Ear Lobe	Nil	Nil
Eye Brow	2 + pos.	Nil
Nostril	1 + pos.	Nil
Forearm	1 + pos.	Nil
Leg	4 + pos.	1 + pos.

Interpretation of Ridley Count:

6 + pos	average of 1000 bacilli per field
5 +	average of 100 bacilli per field
4 +	average of 10 or more bacilli per field
3 +	average of 1 or more bacilli per field
2 +	average of 1 or more bacilli per 10 fields
1 +	average of 1 or more bacilli per 100 fields

The results of the Ridley count help to substantiate the histological finding as to this being leprosy of lepromatous or dimorphous type, as in tuberculoid leprosy these sites are generally negative.

A Morphological Index was attempted but lack of experience made interpretation very difficult and the results were not considered to be reliable and so were not recorded.

Since firm diagnosis was made the patient

has been treated with Rifampicin and Lamprene.

Leprosy is still a common disease in many areas of the world and this case is not presented to add any new information on this disease. It is presented, however, to draw the attention of laboratory technical staff in New Zealand to the ever-increasing possibility of being faced with "tropical diseases" and the need to be conversant with techniques required to diagnose such diseases, which do not normally occur in New Zealand but which with modern travel are likely to occur more frequently.

P. Bryan, ANZIMLT,
September, 1974. Nelson Hospital.

A Rack for Westergren ESR's

The Westergren method of Erythrocyte Sedimentation Rate (E.S.R.) determination of aggregated red cells in plasma has been well established as a non-specific screen for qualitative and quantitative changes in the plasma proteins, e.g., collagen disorders, paraproteinaemias, infections, neoplasms; although a normal E.S.R. cannot be taken to exclude organic disease. Additional factors affect the E.S.R. such as haemoglobin level, age, sex, menstrual cycle, pregnancy, drugs taken (corticosteroids, contraceptive pills, etc.).

With the advent of the Department of Health recommendation that all mouth-pipetting of blood and blood products be abolished because of the hepatitis risk, the E.S.R. method was changed. Formerly glass Westergren tubes with the whole blood citrate dilution were placed in metal racks vertically for one hour.

The ratio of four parts E.D.T.A. anticoagulated whole blood to one part trisodium citrate (31.3g/litre dihydrate salt) was retained with 1ml and 250μl "Oxford Sampler" dispensing pipettes and disposable plastic pipette tips. Plastic "Dis-Pet" Westergren pipettes and blue cups replaced the glass pipettes. It was intended that the blood and citrate be dispensed into the blue cup, the mixture filling the pipette by pushing the pipette into the cup, and standing in the rack provided for one hour.

Two problems arose: the blood-citrate mixture did not reach the zero mark 200mm up from the bottom, and verticality could not be guaranteed.

Figure 1.—Westergren E.S.R. rack (lengths shown in mm).

The solution was to dispense the blood and citrate into a plastic 75mm x 8mm test-tube, push the blue cup fully on to the top of the plastic Westergren pipette, and draw the blood up the pipette by withdrawing the cup the necessary distance. The three (test-tube, pipette and cup), were placed as one unit in the rack made by the hospital carpenters as illustrated in Figure 1. The grooves were made by drilling a series of holes in a block then cutting them in half. The spirit level was situated on this block, the only part of the rack required to be level. The clips for each E.S.R. are aluminium window louvre catches, replacing our original terry-clip suggestion. The comparison of new and original methods gave comparable results.

One word of caution: The Oxford 1ml pipette has a very strong spring, often causing blood to enter the instrument upon sudden release of the plunger, thus blocking it on drying and also causing the plunger to stick, necessitating dismantling and cleaning.

M. W. Dixon, ANZIMLT,
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Auckland.

Help for the Developing Countries

The New Zealand Institute of Medical Laboratory Technology plays an active part in assisting the laboratory services of developing countries particularly in the Pacific area. Gifts of laboratory equipment have been supplied on request whenever a need has been made known. This voluntary service is organised through the Council's Public Relations Sub-committee.

Marilyn Eales, a former Council Member has performed a tour of duty in the medical service of Papua and New Guinea. She has been concerned with the training of medical laboratory technicians in the Faculty of Medicine of the University of Papua and New Guinea, Boroko, Port Moresby. A three-year course is conducted. The Institute was instrumental in obtaining textbooks for the use of the students and the photographs show the interest engendered by their receipt. The books were received in August 1974 and at this time Marilyn wrote formally thanking the Institute Members for the gift on behalf of the Student Technicians of Papua New Guinea.

Correspondence

Sir, I am grateful to those who have not only requested reprints of my paper, 'The Laboratory Diagnosis of Respiratory Infections Other Than T.B.' published through the Journal, Vol. 28, No. 1, 1974, but have also asked a number of questions. I will attempt to answer through your column.

1. The paper was researched within the overall context of bacteriological investigations. Additionally we routinely grade specimens according to their macroscopic appearance. M.P. +++—±.

2. We are presently using the Oxoid BRI buffered pancreatin tablets but have also a high regard for the Calbiochem product 'Sputolysin'. Any such agent which successfully splits mucoprotein linkage without causing damage to delicate or fastidious pathogens is a welcome addition. In my experience the only critism one might level against this product is the slightly reduced number of *H. influenzae* and their tendency to become rough rather than remain capsulated.

3. I agree with the observations of Shah *et al.* (1966) that under some circumstances 'Sputolysin' is preferable to N-Acetyl-L-Cysteine as a digestive agent for the culture of mycobacteria.

4. Greater emphasis should be placed on anaerobic cultures but this fact was not within my terms of reference

5. Additional experience clearly shows that fresh specimens are vital and doubtful results are often clarified with comparative mouth washings.

Further direct correspondence will be most welcome.

HENRY C. W. SHOTT,
Microbiology Laboratory,
Dunedin Hospital.

January 1975

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Sir, — The only conclusion reached about education during the past year or so, is that there is too much talk and not enough action. The matter was even considered a fit subject for levity by some senior members at the last conference. It is not. May I offer some suggestions?

The principle of trainees not being part of the work force should be established. They should serve a stipulated probationary period.

The NZCS should be accepted in its own right and holders might eventually replace laboratory assistants. Incidentally, is there any need to designate the qualification as a New Zealand Certificate of Science? Would Certificate of Science not suffice? This would impart a degree of universality to it.

There is scope for more cross-crediting between the QTA and O, the medical laboratory courses, and university units.

The moves to allow graduates, that is university graduates, to obtain a final certificate of attainment and become registrable should be pursued. Certainly a more appropriate designation than graduate technologist should be found such as Junior Hospital Scientific Officer.

Technologist training and education requires a balance of academic and practical work. A single subject B.Sc. is unsuitable in that the aims and objects are quite different. Not only is there no practical orientation but the vocational aim and motivation are also lacking. Education does not simply consist of imparting theoretical and practical concepts, attitudes are also important.

I favour alternate three-month periods of academic and practical work. The academic work would take place at a separate training centre. Eighteen months of theory and a similar period of practical work in any case would be required to reach an NZCS standard.

The final qualification would be of a specialist nature and the preparation of a thesis or evidence of suitable published work would be a prerequisite. The qualification should be of such a standard as to merit cross-crediting for an honours B.Sc. Here again the need for a suitable balance of theory acquired at a centre and practical work would be necessary.

Michael Legge,
Clinical Biochemistry,
Christchurch Hospital.

November, 1974.

(Abbreviated.—Editor.)

BOOK REVIEWS

Living with Haemophilia Peter Jones, M.D. Published by Medical & Technical Publishers Ltd 1974. Price $NZ13.20. 177 pages with illustrations. From N. M. Peryer Ltd, Christchurch.

In the introduction Dr Jones states that although there are many excellent books on the haemophilias for doctors there are none for haemophilic patients or for the many paramedical workers who help them. He has therefore set himself the very difficult task of presenting a complex subject in a form that can not only be understood, but be of benefit to readers of widely varying intelligence and education.

Overall the author has succeeded admirably in his aim. The book is easily read, with clear headings and abundant illustrations. He frequently uses simple analogies to explain the more difficult facets but sometimes I feel he overdoes this and leaves the reader even more confused. Another mild criticism is that much of it is written in lecture style, punctuated bv the use of local slang.

The book begins with a brief description of the anatomy and functions of the body including an explanation of how bleeding is controlled. Later chapters explain the transmission of the bleeding disorders through families, their manifestations and their management. The final chapters discuss recreation, education, employment and family planning. In brief, therefore, this book touches on all aspects of the haemophiliac's life from the cradle to the grave, and the author emphasises that with proper modern care a haemophiliac can be and should lead an almost normal life. In addition. the Appendix contains a comprehensive list of all drugs containing aspirin (which must be avoided by haemophiliacs), and another list of the safer paracetamol preparations.

One great advantage of this book is that it can readily be ' dipped into ' and, for the paramedical worker, including the medical laboratory technologist, will give added insight into the special problems confronting the haemophiliac.

Although I feel that this book goes a long way in helping to combat the ignorance which produces fear and withdrawal in patients and over protection by parents, any book of this nature does have its dangers. Whereas doctors readily accept controversy in medical management, lay people do not, and this may produce conflict between the haemophiliac who has read this book and his doctor even although the author does repeatedly stress that ideas do differ.

A. G. R.

The Future of New Zealand Medicine. D. W. Beaven and B. H. Easton. Published by N. M. Peryer Ltd. Christchurch, 1974. 150 pages. Price $NZ3.00.

The authors in the acknowledgment to this collection of essays by New Zealand medical personalities, note that during the course of their preparation, Mr N. M. Peryer died suddenly. His enthusiasm and support for this book are acknowledged. In co-operation with the essayists he agreed to donate any profits to the Canterbury Medical Research Foundation. They conclude with the words: ' New Zealand Medicine is the worse for his death and we acknowledge his many contributions during his lifetime '.

Many familiar names appear in the list of contributors and this familiarity adds interest to the book. A wide range of topics are covered and they are loosely arranged under three headings. General; Organisation and Resources; Illustrative Areas. It is a mixed bag and the standard varies somewhat. To use a current metaphor some show a poor signal to noise ratio! However, they are all readable and entertaining.

A recurring theme is the inadequacy of the Health Service. It could be expressed as the persistence of one rule for the rich and another for the poor. The insistence of the right of private practice and the resulting anomalies crop up in at least four essays. In this connection an essay by G. M. Fougere has some disquieting revelations in ' Medical Insurance: The Market's Quiet Counter-revolution '. The basic contention for which evidence is presented, is that medical insurance works to destroy welfare arrangements and that the immediate effect of people taking out medical insurance is to drain off energy and concern for the public sector.

Another thought-provoking report is about Nursing. S. C. and M. S. Thomson purport to

show that trained nurses are wasted in that registered nurses are immediately cast in a supervisory role or as the authors put it, 'the mystical world of administration and supervision for which they claim they have not been trained'. The anomaly is compounded because those actually working at the bedside are the trainee nurses. This seems the classic case of too many chiefs and not enough indians. Another bone of contention is the proposed use of nurses in general practice to act as doctors' assistants. This confuses the role and responsibility of the nurse which is traditionally care rather than cure. It is suggested that this could de-professionalise, under-involve and lower the initiative, autonomy and morale of the nursing resource.

Attention is drawn in other essays to the interdependence of the health team and the need for due recognition of the other health-related professionals in medical treatment. Such people are required to undertake increasingly complex and sophisticated training at technical colleges and universities coupled with inservice educational programmes. An adequate supply of people to staff these complementary professions is inseparable from a consideration of the number of doctors required.

There are twenty-five essays in all and I have merely touched on a few of them. I enjoyed reading them all R.D.A.

Manual of Clinical Microbiology. Second Edition. Edited by Edwin H. Lennette, Earle H. Spaulding and Joseph P. Traunt. Published by American Society for Microbiology, Washington D.C., 1974. Price $US15.

In 1970 the first edition of this Manual was published under the auspices of the American Society for Microbiology as a cumulative effort by 99 contributors in their own special fields, published in a text of some 700 pages. The contributors have now risen to 125, size to 970 pages, and the Manual has has been extensively revised and much new material has been added.

The first General Section has been doubled in size and a useful summary of the position in the United States regarding classification and nomenclature has been added. It is interesting, in this chapter to note the references to the "forthcoming" edition of Bergey's "Manual of Determinative Bacteriology" which has now been some seventeen years in revision. Four practical pathologists have contributed to a large section on the collection, handling and processing of specimens and even if the methods cited are not practicable in all laboratories the suggestions give considerable insight into possible isolations of all potential pathogens from all sites.

The specialist chapters on the aerobic bacteria, spirochaetes and anaerobic bacteria now constitute 30 percent of the total volume. They are written more as the form of a bench manual with a general description, cultural and identification techniques, and discussion of clinical significance and likely isolation of each particular organism.

The chapter on the miscellaneous Gram-Negative Bacteria has been very much revised and the nomenclature as laid down by Lissel in 1971 on the taxonomy of Moraxella and allied bacteria is used. The illegitimate epithets of Mima, Herellea and *Bacterium anitratum* have been discarded at last and replaced by the genus Acinetobacter and the species Calcoaceticus. The authors recognise now the two further varieties or subspecies *A. calcoaceticus* var. *anitratus* and var. *lwoffi*. Detail is also provided in the difficult field of the Pseudomonas-like and the Moraxella-like organisms.

Laboratory tests in chemotherapy deal with the accepted methods of testing by diffusion, tube dilution and assay of agents and each section outlines pitfalls and common errors inherent in each method. A very quick summary at the end of the section looks at future needs in this field and makes some thought-provoking statements.

Immunoserological tests are dealt with briefly, 15 pages in total, but the introduction to the Manual asserts that this is due to the fact that the Society is to shortly publish a companion Manual of Clinical Immunology.

Fungi, from an introduction to Clinical Mycology through the agents of superficial mycoses, yeasts, Aspergilli, etc., is a comprehensive section and concludes with two chapters on serodiagnosis of fungal disease and susceptibility testing of antifungal agents. It is interesting to note that the authors

Ajello and Padhye still use the older terms of macro- and microcomidia as opposed to the botanical based and more precise terms macro and microaleurisospores. This reviewer is much in favour of the older terms from the simple point of pronunciation and ease of conversation!

A comprehensive section deals with parasites through the gambit of intestinal protozoa, soil amoeba, blood and haemopoietic parasites, helminths and arthropods affecting humans. This section includes some very good colour reproductions of the plasmodia and the illustrations of the eggs of the helminths have now been included as microphotographs rather than the less impressive line drawings of the previous edition.

Section IX of some 160 pages deals with viruses and Rickettsiae from tissue culture methods up to a description of the radioimmunoassay methods for Hepatitis B antigen.

The penultimate section of Infection Prevention—Quality Control replaces the older and non-specific section labelled Miscellaneous Procedures. Dr Bartlett heads the contributors on the control of hospital associated infections dealing with this problem under the subheadings of infection surveillance and control and microbiological surveillance.

A short chapter deals very succinctly with bacterial food poisoning and Dr Ruth Russell of the Memorial Hospital Medical Centre, Long Beach, continues her battle for an increasing awareness and use of quality control programmes in the microbiology laboratory.

Section XI deals with media, reagents and stains that are used or referenced in the main text. Apart from formulae there are also listed some pertinent facts as to the use and performance of some of the reagents and stains listed.

This is a very comprehensive and well set out manual. The authors were briefed to produce a " working and teaching guide that will be of practical use . . . " and in this respect they have succeeded. In common with the twelfth edition of Medical Microbiology they have produced a soft-covered binding—two choices, cloth or plastic—and I only wonder if this material is robust enough to survive the daily use on the workbench.

This is a book that should be available to all work-benches in microbiology sections as it is up-to-date and references are there with each chapter to encourage any further reading.

A comparison with the twelfth edition of Medical Microbiology is not pertinent as the second section of Medical Microbiology is not yet available and the Manual of Clinical Microbiology does not deal with the fundamental teaching of anatomy, morphology, etc., of the bacterial cell.

I would recommend that all candidates for Parts II and III examinations in microbiology should have the Manual of Clinical Microbiology available as one of the standard texts.

—M.D.M.

Computer Technology in the Health Sciences. David B. Shires. 1974. Charles C. Thomas, Publisher. 140 pages, illustrated. Price $US10.75.

The aim of this book is stated " to provide the novice health professionals with an awareness and a framework for applying computer technology to the health field ". Its author is from a University Division of Family Medicine, and from his vantage point, he commands a wide view, from the family doctor's clinic to the sophisticated hospital department.

It is about general applications of computers, establishing a case for maintaining computerised health records for the whole populace, based mainly on the cost savings accruing from ease of access, and from the resulting improved patient care arising from better information. This type of system is then examined chapter by chapter from the aspects of systems work, health data coding, record linkage, legal problems, service applications and education. He also includes chapters on basic jargon, Medical Information Technology and a glimpse into the future. By the nature of the book, each aspect is covered broadly, happily never getting bogged down in details (the laboratory computer gets two pages!), but most of the relevant questions are raised. However, in light of recent events here, one would have liked to see the confidentiality of health records stressed much more fully. Also, in the last chapter the point of humans controlling machines and not vice versa could have been made with a good deal more subtlety.

However, the author must be praised for the readability of a text on a usually very dull topic and for the glimpses of a very human approach to the subject. For instance he formulates three laws of computing: "(a) if a task can be done in any other reasonable way, avoid using a computer, (b) if a computer can possibly be blamed for a human error, it will, and (c) the information content of an output is inversely proportional to its weight." But for all that, one could not help feeling that his initial dispassionate distance from his subject slightly diminishes as he progresses.

As an introductory text, this book fulfils its purpose since it can be assimilated on first reading, and I would recommend it as useful background reading for aspiring laboratory administrators and for anyone else who is required to keep the role of the computer in his specific application in perspective.

M.A.

Clinical Laboratory Methods. John D. Bauer, Philip G. Achermass, Gelson Toro. 8th Edition, July 1974. Published by C. V. Mosby Company Ltd. Price $NZ20. N. M. Peryer Ltd, Christchurch.

This book has eight chapters relevant to the *chemical pathology* laboratory.

Chapter one "some laboratory rules and quality control" gives basic information on the causes of laboratory errors, and on quality control.

The chapter on urinanalysis has about 40 pages devoted to urine tests and goes into some detail in describing methods to identify specific proteins. Inborn errors of metabolism are well covered, some 13 pages being devoted to this. The remainder of the chapter is devoted to haemoglobin pigments, porphyrins, renal function tests and microscopy. The latter section contains 11 pages with numerous illustrations.

The major chapter devoted to clinical chemistry has a thorough introduction describing principles of analytical techniques, e.g., atomic absorption spectroscopy, fluorimetry, etc., and the evolution of automatic analysers. Simple pipetting apparatus are described and such machines as Technicons, "S.M.A.C." and Union Carbides "Centrifichem". In the introductory section units in general use and S.I. units are discussed. The analytical methods are briefly preceded by the general principle of the test and its clinical use. The chapter includes some recent methodology, e.g., Fredrikson's classification of lipoproteins, and osmolality measurement, although the section on blood gas analysis describes the use of a Natelson micro gasometer and only brief mention of electrodes.

A chapter devoted to enzymology has an introduction discussing units in use and principles of measurement. Each enzyme is listed by its proper name and E.C. number.

Hormone analysis is introduced by a section on analytical technique, e.g., competitive protein binding, and radioimmunoassay. This chapter of some 28 pages covers the commonly measured hormones.

A chapter on gastric and duodenal analysis covers the tests in common use including tests for fibrocystic disease.

The final chapter relevant to clinical chemistry describes methods in toxicology. Such tests as alcohol, arsenic and barbiturates have specific methods described. A section on drug screening devotes some seven pages to chromatography procedures.

I would conclude that this book would be a useful addition to a chemical pathology laboratory for as well as the information included in the book each chapter has a comprehensive list of references.

Haematology Section, chapters 4-7, pages 85-291, 216 pages.

The material in this section offers a refreshing change from some recent books of this type in that it is for the most part up to date and presented in concise and readily assimilated fashion.

The four chapters cover general methods of blood collection and haemoglobins (normal, abnormal, estimation electrophoresis, etc.), evaluations of formed blood elements (cell counts, differential counts, cytochemical stains, cell abnormalities), methods for evaluating red and white cell pathology (anaemias, leukaemias, haemoglobinopathies) and methods in coagulation disorders.

Methods given are often brief or only outlined but an excellent system of references is provided for obtaining additional information. In some instances these methods are based on commercial kitsets or reagents which are in common use.

The coagulation section is up to date and includes methods for fibrin degradation products.

It is rather curious to see methods for deter-

mining oxygen saturation and carboxyhaemoglobin levels in a haematology section though the former has relevance for the standardisation of the haemoglobin estimation. The authors however still advocate the use of the Wintrobe ESR technique which is not recommended by the International Congress of Haematology.

Finally there is a useful glossary of terms at the end of the section.

Six years have elapsed since the previous edition of this valuable manual. The sections related to *Microbiology, mycology and parasitology* have been revised and are remarkably up to date. The authors should be especially commended for the manner in which they have brought the virology methods within the practical reach of the non-specialist. Table 16-45 which lists the diagnostic tests applicable to the particular virus has done much to help those working in the smaller laboratories. From the teaching point of view the bacteriology layout states the principle of most of the less common tests and offers sensible tables to produce alternative classifications, particularly in the area of the enterobacteriaceae. Perhaps the Kirby-Bauer sensitivity testing section may have better emphasised the difficulties of producing accurate methicillin results and also have recommended that the sensitivity of *Streptococcus pneumoniae* to penicillin is less predictable at this time. Both the mycology and parasitology chapters have the traditional approach which means they are the more easily read. In all a timely addition to a good series.

The section on *blood groups,* etc. (chapter 8) gives a relatively wide coverage of ABO and Rhesus groups for a general text book. The terminology may cause some confusion amongst junior trainees as it follows American thoughts rather than the European which is in more general use in New Zealand. This is particularly so in the section on Rhesus groups where Wiener's nomenclature is used.

Other blood group systems receive only scanty mention, and the section on Lewis groups includes material that is not generally accepted. Some of the more important "public antigens" are not listed at all.

The techniques given would suffice to provide an elementary blood grouping and compatibility testing service, but gloss over some problems that should be known by any technologist who is in charge of a blood bank.

Overall, the chapter on blood group serology is adequate for a volume of the general nature of this publication, but falls well short of the information needed on the subject for N.Z.C.S. Any trainee would be well advised to refer to more specialist volumes on the subject.

—D.S.F., B.W.M., H.C.W.S. and A.G.W.

Microanalysis in Medical Biochemistry. I. D. P. Wootton, 1974. 5th Edition. 307 pages. Copy supplied by Penguin Books (NZ). Price $NZ10.90.

This new edition of a standard laboratory textbook will be welcomed as it is ten years since the previous edition was published. Its function to describe the techniques currently in use at the chemical pathology laboratory at the Royal Postgraduate Medical School and to provide alternative manual methods for smaller laboratories has been retained. Although much of the material has been updated the book still remains a convenient size (14 x 22 cm) to be of practical use.

The format has been retained in that each chapter is introduced by a brief description of the clinical use of the method, the chemistry involved, and the methods in current use. Usually two or three methods are described, for example, Bilirubin is described by direct spectroscopy, diazo reaction manually, and by Technicon autoanalyser method.

As one would expect the use of autoanalyser methods is more widespread than in the previous edition and flow diagrams are given. A departure from the previous edition is the inclusion of references at the end of each chapter.

New material appears in almost every chapter. The chapter entitled 'Normal or Reference Values' describes current quality control procedures such as the use of cusum charts and the calculation from patients' results of the truncated daily mean. Of particular interest is the chapter on automated analysis which describes the principles of the Technicon autoanalyser. A very useful section on fault finding has diagrams of autoanalyser charts accompanied by explanation of the faults. Autoanalyser II and SMA systems are only briefly mentioned and the only discrete

analysers mentioned are the Vickers D.300 and the L.K.B. 8600.

The section on enzymes has been enlarged and the chapter introduced with a discussion on the international unit and the possible introduction of the 'Katal' unit in the S.I. system. Each enzyme is listed by its common name, true chemical name and E.C. number. Most enzymes have more than one analytical method, for example, 2 point colorimetric, U.V. method, or by autoanalyser.

Another section which has been expanded is the chapter on Special Constituents and Drugs. Drugs commonly encountered in overdose situations are described, for example, barbiturate, methaqualone, paracetamol, salicylates, iron, and bromide. Particularly useful is the procedure given for the detection of basic drugs in gastric aspirates and the tables in the chapter giving information about drugs.

One of the new chapters is entitled 'Blood Lipids'. Autoanalyser methods are described for cholesterol and triglycerides in addition to manual methods. A method is described for lipoprotein fractionation but the widely used Fredrickson classification is not.

An old-fashioned technique is described in the chapter on c.s.f. where Lange colloidal gold reaction is described. No mention is made of a specific gamma globulin method.

The balance of the book has a chapter on electrolytes, proteins, steroids, identification of sugars and amino acids by chromatography, function tests and a good section on qualitative tests for urine and faeces.

The concluding chapter Volumetric and S.I. Units details the changes which will occur in the author's laboratory when S.I. units are introduced. Useful tables are given.

Because this textbook contains comprehensive information it will remain an invaluable basic bench book in any chemical pathology laboratory.

A.G.W.

Clinical Haematology. Fourth Edition R. D. Eastham, B.A. (Cantab), M.D (Cantab), D.C.P., Dipl. Path., F.R.C. Path. 275 pages. John Wright & Sons Ltd, Bristol 1974. Price in U.K. £1.60.

The Fourth Edition of Eastham's Clinical Haematology has almost reached the size where it will no longer conveniently fit into a laboratory coat pocket. This is due to the addition of some 70 pages since the third edition thus increasing its thickness to 13/16 inch.

One major increase is in the coagulation section with the addition of 23 pages. Platelet function and coagulation factors are more extensively covered and there are expanded sections on FDP's, Complement and Anticoagulant therapy.

Sections on Haemolytic anaemia and Megaloblastic anaemia also show extensive revision and there are new areas dealing with Haemopexin and Dyserythropoietic anaemia.

The format of earlier editions is unchanged so that information can be readily found under the various headings. This is an invaluable book both for students and specialists in haematology, and those who have the old edition are advised to obtain the new one.

B. W. M.

A Short Textbook of Medical Microbiology. D. C. Turk and I. A. Porter. Unibooks. English Universities Press, London. 3rd Edition 1974. Price, £UK3.45 (Boards), £1.85 (Unibook).

The 332 pages of this soft-covered book span a wide range of aspects of medical microbiology in a succinct yet readable form.

It presents an account that would be of interest and help to medical practitioners not especially trained in microbiology and to students who wish to obtain a general rather than a specialised knowledge of medical microbiology.

It does not present, nor does it pretend to present, a detailed account of specific laboratory methods and its appeal to technologists, who are required to have a knowledge of practical procedures, will therefore be somewhat limited.

N.P.M.

Review of Medical Microbiology. Ed. by E. Jawetz, J. L. Melnick, and E. A. Adelberg. Lange Medical Publications, 11th Edition, 1974. 528 pages. Price, $NZ7.65. N. M. Peryer, Christchurch.

Like its predecessors this 11th edition presents up-to-date information in a precise manner. Basically, this is no longer a 'Review' but a standard textbook which should be strongly recommended in any microbiological teaching situation. The authors have never pretended that they offer a technical textbook otherwise one could say that lack of laboratory methods was a grave disadvantage.

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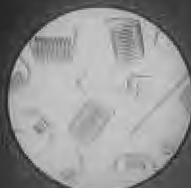

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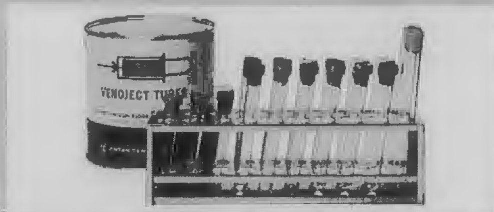

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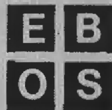

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However it does state the line of investigations which may be taken and clearly expects the technical development to be found elsewhere. The undoubted strength of the edition is its balance. For example the section on viruses is almost a textbook in itself and chapter twenty-seven covers the properties of viruses in a most competent manner.

Recent advances in microbiology generally have demanded that our ideas on cell structure and cell biochemistry be modified and these are illustrated with clarity and concise definitions.

The host relationship to pathogens is covered well in chapter eleven although the present-day concepts of the 'compromised host' might well have been more adequately stressed.

As usual the section on parasitology is simply and well illustrated getting at the real laboratory needs with as few words as possible. Antimicrobial chemotherapy shows an American bias, is excellent except more regard might have been shown to the relationship between dosage and protein binding, also more emphasis should have been placed upon laboratory control. The mycology section, again deals exclusively with pathogens, whereas the laboratory still has to cope with misleading contaminants.

In all, for its purpose, a concise edition dealing mainly with theoretical aspects, this revised edition is a valuable addition to any bookshelf.

H.C.W.S.

Fundamentals of Microbiology. Ninth Edition, 1974. Frobisher, Hinsdill, Crabtree and Goodheart. 850 pages. Published by W. B. Saunders Company, Philadelphia. Price, $NZ14.85. N. M. Peryer Ltd., Christchurch.

If enthusiasm is infectious, readers of 'Fundamentals of Microbiology' will all become enthusiastic about microbiology. This extensive survey of basic microbiology for university students is so attractively presented, and is garnished with such glamorous career suggestions, that a career in microbiology must become irresistible. Organisation of the text makes it an efficient reference book. Noteworthy features include the treatment of individual topics under separate headings, the use of heavy type for all new terms and a comprehensive index. There is also a glossary, but it does not adequately cover the technical terms in the text. However, the illustrations, charts, diagrams, graphs, photographs and electron micrographs, are excellent. They not only explain but extend the text. Nonetheless, it is puzzling that full page electron micrographs merit acknowledgments but no captions.

The style of writing is lucid. The experience of the authors and their skill as teachers is reflected by the clarity of explanation and the absence of unnecessary technical language.

Section 1 is introductory. It describes the development of microbiology and its relationship to other sciences. It is very up-to-date in its coverage of such diverse topics as evolution, scanning electron microscopy, x-ray microscopy and the chemical basis and origin of microbial life.

Cell structure, fungi, algae, protozoa, viruses and genetics are included in Section 2. Viruses and genetics, two confusing subjects for junior students are particularly well described. In this section, as in the other four, limitations in present knowledge are emphasised and explained where necessary.

Section 3 describes micro-organisms and their environment. This leads into sterilisation, disinfection, antibiotics, infection and immunity.

A preview of a new system for the classification of bacteria used in the 8th (1974) edition of 'Bergey's Manual of Determinative Bacteriology' is found in section 4. It is compared with the system for the classification of bacteria used in the 7th (1957) edition of the same manual. While a comparison of both systems is academically interesting it is unnecessary and confusing in an introductory textbook. It would seem logical to use the most recent system.

Section 5 describes micro-organisms in industry and the environment. In the light of present day concern about the environment, diminishing natural resources and ever-increasing consumer requirements the emphasis given to these topics seems to be both practical and justified.

Lists of supplementary reading are provided at the end of each chapter. Usually they consist of general reviews, monographs and books suitable for junior students. There is also a companion laboratory book.

This book gives a comprehensive coverage of the fundamentals of microbiology. It would be a suitable book for the university student fol-

lowing a general course in microbiology, but not so appropriate for the medical laboratory technologist. Microbiology of medical importance is only covered as an illustration of general microbiological principles. With this proviso, this should be a stimulating textbook for students of microbiology or for any microbiologist wishing to study a new topic. R.M.

Clinical Chemistry and Automation. A study in laboratory proficiency. Ronald Robinson, B.Sc., Ph.D., F.R.I.C. 1971. Published by Charles Griffin & Co. Ltd., London. 187 pages, illustrated. $NZ7.15.

An important aspect of automation is that human fallibility is largely avoided although no one would claim that automation is free from error. The tedium of performing repetitive tests results in increasing errors and although this will cause little surprise amongst laboratory practitioners the matter is put beyond doubt by quantitative experiments described in this book. Ironically the conscientious individual by unrelenting concentration is likely to perform worse than a less dedicated person. Where such tests have still to be carried out it is well to remember that appropriate rest periods will reduce error. Certainly it is essential that laboratory heads consider the stresses and strains to which the staff are subjected and act accordingly. The use of simple automatic devices will facilitate matters but heavy work loads demand a high degree of automation.

Although in the future the accuracy of laboratory tests may depend on the care with which a volume of water is pipetted into lyophilised serum, automated equipment is not yet foolproof. Mechanical faults can develop and published methodology may still be susceptible to improvement. Various discrete and continuous systems are discussed and comparative data on their performance is tabulated. The simplicity of the continuous flow methods are contrasted with the enormous speed potential of the discrete systems.

Once results start to pour out, bottlenecks may develop in assembling and reporting the data and some means of automatic data processing is required. Two chapters are devoted to discussing data processing and the laboratory. Potentially there are many bonuses from computerisation. Quality control statistics, estimation of normal ranges, frequency of abnormal results and the preparation of cumulative reports are possible. The need for a quality Control Officer is stressed. Quality Control results should not be rationalised out of existence; appropriate action is required. Most laboratories who have appointed a Control Officer would most heartily agree that a competent person of sufficient status will increase and maintain the precision of the laboratory results.

The interpretation of laboratory results, and screening and biochemical profiles are also dealt with.

I was sufficiently interested in the subject matter of this book to acquire a copy from overseas. I was not disappointed. The author has marshalled the facts and illuminated the problems currently associated with clinical biochemistry in a most succinct fashion. Many of us working in the field may well feel a sense of gratitude to him for having done this.

R.D.A.

Clinical Chemical Pathology, seventh edition, 1974. C. H. Gray. Published by Edward Arnold, London. 247 pages, illustrated. Price, £UK4.50 (boards), £UK2.25 (paperback).

The first edition of this little book came out in 1952 and it has been followed by further editions and reprintings at regular intervals since. This could be regarded as the SI Unit version and it is a timely innovation for us in New Zealand as it finds us trembling on the brink of this important step. The introductory chapter provides a brief exposition of the advantages which might be useful for purposes of quotation. The most cogent argument at this stage is probably that everybody's doing it.

The chapters on liver function, diabetes, acid-base and plasma protein have been rewritten and minor changes can be detected in other parts of the book. The chapters on the nervous system and clinical enzymology have been deleted and the essential information redistributed. By this means the book is virtually the same size as the previous edition.

The acid-base chapter uses SI Units and a graph indicates the relationship between pH and $[H^+]$ expressed as mmol/litre.

Current information about α_1 antitrypsin and α_1 foetoprotein appear in the protein chapter

and relevant information about the B and T lymphocytes.

An up-to-date view of diabetes and the various mechanisms of the diabetic syndrome are presented. Another innovation is an appendix of routine and special tests. There is also a brief summary of normal values and an indication of abnormal situations.

The condensed style achieves the object of presenting a concise account of clinical biochemistry. Those of us who are confronted with the information explosion in the current literature will realise how difficult this is. Its popularity is well deserved.

R.D.A.

Abstracts

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

Clinical Biochemistry

Performance of 'Kits' used for the Clinical Chemical Analysis of Salicylates in Serum. Kim, H., Skodon, Sandra B. and Barnett, R. N. (1974), *Am. J. clin. Pathol.* 61, 936.

The use of 'Kits' for the chemical analysis of serum salicylates has been evaluated. Eleven 'Kits' were tested and compared with a reference method. Only five of the 'Kits' gave acceptable results and those using Trinders reagent performed better than the others. —L.R.F.

The Size and the Source of Analytical Error in Clinical Chemistry. Gilbert, R. K. (1974), *Am. J. clin. Pathol.* 61, 904.

Data from the College of American Pathologists' Survey Program have been analysed. From the article, 'The implications of the survey all point in the same direction. The data suggest that far more attention should be paid to precision and internal quality control programs. The data also suggest that improved precision may best be achieved by changing methods. The survey cannot provide full information on many aspects of a method, such as method specificity, but it does suggest that the accuracy of a method may be less important than its precision.'

—L.R.F.

An Evaluation of Clinical Laboratory Performance of pH-Blood Gas Analyses Using Whole-Blood Tonometer Specimens. Weisbrot, I. M., Kambli, V. B. and Gorton, L. (1974), *Am. J. clin. Pathol.* 61, 923.

From the article 'Tonometrically calibrated blood specimens demonstrated that blood gas analyses are subject to small systematic errors (biases) and that precision may be poor when analyses are performed under conditions simulating analysis of patient samples. This is in contrast to performance of pH-blood gas instruments under ideal laboratory conditions'.

The investigations of this study show that there is need for quality control surveys for pH-blood gas analyses and a need for stable synthetic reference materials which simulate blood. —L.R.F.

Specific-Automated Method for Measurement of Urinary Hydroxyproline. Seymour, G. L. and Jackson, M. J. (1974), *Clin. Chem.* 20, 545.

An automated continuous flow method is described which utilises column chromatography and sulphonated polystyrene resin. The eluate is reacted with chloramine T and p. dimethlyaminobenzaldehyde to produce a coloured complex that is measured at 560 nm. —A.G.W.

New Method for Measurement of Guanidosuccinic Acid in Serum. Grof, J., Tanko, A. and Meryhart, J. (1974), *Clin. Chem.* 20, 574.

This compound found in normal serum is attributed to the development of uraemic toxaemia. A modified method is described utilising ion exchange column to remove interfering quanido compounds and elution of the compound for reaction with Sakaguchi reagent. —A.G.W.

Quality Control System for Blood pH and Gas Measurements with Use of a Tonometered Bicarbonate-chloride Solution and Duplicate Samples of Whole Blood. Noonan, D. C. and Burnett, R. W. (1974), *Clin. Chem.* 20, 660.

A procedure is described for control of pH, PCO_2 and PO_2 measurements using solutions of NaCl (150 mmol/l) and Na HCO_3 (50mmol/l). These solutions are equilibrated at room temperature (24°C) with a gas of composition 21% O_2, 12% CO_2 and 67% N_2. On analysis at 37°C the pH should be 7.20, PCO_2 110 mmHg and PO_2 170 mmHg.

The authors show how these solutions can be used for daily control of blood gas measurements and as a possible means for calibrating blood gas apparatus. —A.G.W.

Significance of Bicarbonate for the Evaluation of Non-Respiratory Disturbances of Acid Base Balance. Rispins, P., Zylstra, W. G. and Van Kampen, E. J. (1974), *Clin. Chim. Acta,* 54, 335.

The authors discuss the various parameters used in assessing respiratory and non-respiratory disturbances. They describe how the actual bicarbonate can be used to assess a non-respiratory disturbance. The article is a useful review of acid base balance.

—A.G.W.

A Simplified and Rapid Enzymatic Method for Determination of Urinary Oxalate. Hallson, P. C. and Rose, G. A. (1974), *Clin. Chim. Acta,* 55, 29.

A method using the enzyme oxalate decarboxylase is described. The released CO_2 is taken into an

alkaline buffer and the pH change measured. No preliminary treatment of urine is required and inhibition by phosphate and sulphate are overcome by increasing the enzyme concentration.

Although the method is simple an 18-hour incubation is required. —A.G.W.

A Simple Automated Method for the Determination of Bicarbonate in Plasma or Serum. Den Boer, N. C., Mantel, P. A. and Leynse, B. (1974), *Clin. chim. Acta,* **55,** 87.

An automated method is described which presents results in digital form in mmoles/l. The method is based on a titration technique. After the measurement of sample pH, a standardised excess portion of acid is added to the sample and the CO_2 expelled completely. The excess of acid is titrated with alkali until the original pH of the sample is reached. The difference (in mmoles) between the acid added and the alkali needed for titration is equal to the amount of HCO_3 (in mmoles) in the sample.

The hardware used in the machine's construction which processes 30 samples/hour is described.

—A.G.W.

Evaluation of Some Commonly Used Semiquantitative Methods for Urinary Glucose and Ketone Determinations. James, R. C. and Chase, G. R. (1974), *Diabetes,* **23,** 474.

The 2 drop Clinitest, 5 drop Clinitest, Tes-Tape and Keto-Diastix semiquantitative urinary glucose methods were evaluated on 300 urine samples from diabetic patients and comparing the results with those obtained by an auto-analyser glucose oxidase method. At high levels of glycosuria (above 1,500 mg/dl), the 2 drop Clinitest method gave better quantitation than the other methods. In this range the Keto-Diastix method often gave falsely low results. At intermediate levels of glycosuria (376 to 1,500 mg/dl) there appeared to be little or no difference among the methods. The 2 drop Clinitest method was often insensitive to levels of urinary glucose below 376 mg/dl. In this range the other three methods gave comparable results, with the exception that Tes-Tape was sometimes positive with normal levels (1 to 15 mg/dl) of glycosuria. Proteinuria and pregnancy had no effect on any of the methods. Acetest was a more sensitive and accurate indicator of urinary ketone levels than Keto-Diastix.

—J.H.

Analysis of Proteinuria in Health and Disease Using Sodium Dodecyl Sulphate-Acrylamide Gel Electrophoresis. Mulli, J. C., Balant, L., Giromini, M. and Fabre, J. (1974), *Eur. J. clin. Invest.,* 4, 253.

When sodium dodecyl sulphate (SDS) reacts with a protein, a negatively charged SDS-protein micelle is formed. If the resultant SDS-protein complex is subjected to electrophoresis in acrylamide gel its distance of migration correlates well with the molecular size of the protein, without being influenced by its electrostatic charge. This method has been found to provide a clear and rapid differentiation of tubular from glomerular proteinuria.

The authors found in this study of 17 healthy subjects and 92 patients with various kidney diseases that this kind of electrophoresis allowed a correct diagnosis of the site of renal involvement in 83 per cent of the cases. Predominantly low molecular weight (MW) protein excretion correlated well with tubular damage. Middle and high MW patterns correlated with glomerular disease, and a relationship between nephrotic syndrome with minimal glomerular changes and middle MW pattern was found in two cases, suggesting that the method could be used to evaluate the selectivity of proteinuria. However, the estimation of selectivity by this method should be studied in a sufficient number of cases and compared with measurements of selectivity by the protein clearance method. —J.H.

Free Glycerol Interference in Triglyceride Determination: Warning of a Possible Problem. Donabedian, R. K. (1974), *Letters to the Editor, Clin. Chem.,* **20,** 632.

The correspondent describes how activated zeolite with Lloyds reagent may not remove free glycerol when isopropanol instead of chloroform is used for the extraction. The danger exists when old sera or freeze dried sera which may be used as standards because they may contain as much as 5 mg/100ml of free glycerol due to the breakdown of tryglyceride. This amount of glycerol would correspond to approximately 50 mg/tryglyceride in the analysis.

This source of error could be solved by processing blanks, a procedure seldom done.—A.G.W.

Use of the Simplex Method to Optimise Analytical Conditions in Clinical Chemistry. Krause, R. D. and Lott, J. A. (1974), *Clin. Chem.,* **20,** 775.

The optimum analytical conditions with regard to two or more variables can be determined in an unambiguous way by use of the simplex method. The method is particularly applicable to problems involving interacting variables. The authors describe its use on two autoanalyser methods. —A.G.W.

Effect of Venous Occlusion of the Arm on the Concentration of Calcium in Serum, and Methods for its Compensation. Hudson, H., Rapoport, A., Locke, S. and Oreopoulos, D (1974), *Clin. Chem.,* **20,** 529.

Data are presented on the effects on 12 normal subjects of a 3-minute period of venous occlusion of the arm. Total calcium and total protein are significantly raised while Mg, PO_4 and ionic calcium are unchanged. The methods used to adjust the total Ca from protein concentration because of venous occlusion are discussed as well as the clinical usefulness of such procedures. —A.G.W.

Network Analysis — A Manual Semi-Automated System for Running Chemistry Panels in the Smaller Laboratory. Amsbaugh, F. D. (1974), *Lab. Med.,* 5 (9), 40.

The author describes a highly efficient system which he has termed Chem/Econ, used successfully by him for over one year. Semi-automated equipment such as automatic pipettes and dilutors are used in combination with a spectrophotometer having a flow-through cuvette and print-out.

Chem/Econ employs network analysis techniques to determine the total time for each test to be performed, free time for each test and the critical path. Free time is that available during a specific test — for example, during incubation—during which the technologist is free to apply time to another test. The critical path through a network of tests is the chain of activities which consume the most time from

the first event to the last. The number of tests a laboratory desires in its chemistry panels is limited only by its needs. Using network analysis techniques, the system offers complete flexibility and selectivity in choosing tests and methods.

A 7-test panel is chosen for illustration, including T_3 aminotransferase, uric acid, creatinine, urea nitrogen, cholesterol and glucose. The flow sheet enables one technologist to process as many as 10 sera (70 tests) plus standards and controls in 2h and 25 min, very economically.

There is still much room for improvement in data recording, operator error, reproducibility and cost. Nevertheless, semi-automatic systems based on the simple principles involved in network analysis techniques help bring efficient, effective and economical multiple chemical testing within the reach of any laboratory. —J.H.

HAEMATOLOGY AND IMMUNOHAEMATOLOGY

Automation of Quantitative Methods in Immunohaematology. Monnet, A. and Cabadi, Y. (1973), *Vox Sang.* **26**, 238.

Application to ABO System: The authors describe an automatic method of determining the percentage of red cell agglutination using specific antisera. Using a continuous flow system this method has been useful in studying population genetics. Comparing automated and manual results, it would appear that this method is satisfactory. It seems probable that this method could be adapted to other aspects of immunology. —L.M.M.

Reliability in Automatic Determination of the ABO Group by the Groupamatic System. Garretta, M., Muller, A., Gener, J., Matte, C., and Moullec, J. (1973), *Vox Sang.* **27**, 141.

The article discusses the reliability of the determination of ABO groups using an automated technique. The reliability depends on three necessary actions of the machine: the identification of the sample, reading serological reactions, processing and printing out grouping data. Facts and figures confirming the reliability of the system are based on the detection of weak A sub-groups, tests on samples of chimeras and Bombay phenotypes.—L.M.M.

Antiglobulin Test in Low-ionic Salt Solution for Rapid Antibody Screening and Cross-matching. Low, B. and Messeter, L. (1973). *Vox Sang.* **26**, 53.

A modification of the conventional antiglobulin test for routine use is described. Saline as the red cell suspension medium is substituted with salt solution of low ionic strength. This permits a considerable decrease in incubation time, which is of practical importance in clinical work. The sensitivity of the reaction does not seem to be decreased by this procedure, in some instances even an increase in reaction strength can be observed.

—Author's abstract.

HL-A Antigen Frequencies in Normal Blood Donors, Kidney Donors and Prospective Kidney Recipients. Nelson, S. D., Darke, C. and Tovvey, G. H. (1974), *Tissue Antigen,* **4**, 361.

The HL-A antigen frequencies in kidney donors, prospective recipients and blood donors are compared. It was found that W28 occurred more frequently in prospective kidney recipients than in the donors. This may be due to the fact that a number of the kidney recipients tested, suffered from glomerulonephritis: it is thought that changes in frequency may be an effect of disease, rather than a cause. —L.M.M.

Recipient's Hepatitis, an Inevitable Side-Effect of Blood Transfusion. Fielder, H. (1974). *Vox Sang.* **26**, 368.

The transfusion of blood with no detectable amounts of HAA, may still be infective for hepatitis A or B. It is thought that a certain unknown number of cases of post-transfusion hepatitis, are due to causes other than blood transfusion. It would appear that in some cases where the disease manifests itself, it is due to the patient being temporarily susceptible, rather than due to a small virus dose. It would seem, that when patients are undergoing surgery, they may have an enhanced susceptibility to hepatitis B. —L.M.M.

Another Example of Anti-D in a D-Positive Patient. Early, M. E. (1974), *Am. J. Clin. Pathol.* **61**, 188.

A case of a woman whose erythrocytes typed as Rh-positive although her serum concurrently contained anti-Rh anti-body is described. It is suggested that the antibody represents iso-antibody to Rh (D) positive blood transfused two months previously. A weakly positive direct Coombs reaction raises the possibility of an auto-antibody.—Author's abstract.

T- and B-Cells in Peripheral Blood During Infectious Mononucleosis. Enberg, R. N., Eberle, Betty J. and Williams R. C., Jr (1974), *J. inf. Dis.,* **130**, 104.

The specific role of increased numbers of circulating lymphocytes in mononucleosis is not well understood. Recent studies in animals and man have allowed classification of lymphocytes into B-cells, bone marrow derived lymphocytes bearing surface Ig and destined for antibody formation, and T-cells, originating from the thymus, showing no readily detectable surface Ig determinants and involved in cell-mediated immune reactions. Attention has been called to the fact that the Downey cell is morphologically similar to the blast cell resulting from the transformation of a T-lymphocyte in response to various plant mitogens, particularly phytohaemagglutinin. A recent report suggests that atypical lymphocytes in infectious mononucleosis are derived from T-cells. Thus the generation of atypical lymphocytes during infectious mononucleosis suggests potent stimulation of the T-cell system. However, immunoglobulins, which are products of B-cells, are often increased in patients with infectious mononucleosis, indicating an intense stimulus to B-cell function as well

The author's data from six patients with infectious mononucleosis suggest that both the T- and B-cell systems were strongly stimulated in this disease.

B-cells in peripheral blood were enumerated by direct surface immunofluorescence and monospecific conjugates for IgG, IgA, and IgM. T-cells were enumerated by two methods. Indirect immunofluorescent staining of freshly isolated lymphocytes utilised rabbit antiserum to human thoracic duct cells, exhaustively absorbed with cells from patients

with chronic lymphocytic leukaemia, who were used as a source of B-cells. In addition, the technique of E-binding or rosette formation between lymphocytes and sheep erythrocytes was used in parallel for enumeration of T-cells. Sheep erythrocyte rosette-forming lymphocytes are considered to represent a population of T-cells. —J.H.

Platelet Electrophoresis: The Present Position. Hampton, J. R. and Mitchell, J. R. A. (1974), *Thromb. Diath. haemorrh.*, **31**, 204.

Measurements of platelet aggregation and adhesion have proved disappointing in the study of individual subjects with, or at risk from, arterial disease. The authors have shown that platelet electrophoretic behaviour can be used to identify individual subjects with coronary artery disease, for the sensitivity to adenosine diphosphate of the platelets of such individuals is ten times greater than in normal subjects. It seems possible that the platelet abnormality precedes the development of arterial disease. The authors suggest that there are changes in the surface-active, or detergent properties of the lipoproteins in subjects with, or at risk from, arterial disease.

The rate of migration of the platelets was found by timing their transit over a given distance using a microscope. The horizontal capillary apparatus designed by Bangham *et al.* (1958) was used.

—J.H.

Heparin Therapy. Raich, P. C., Hahn, M. F. and Korst, D. R. (1974), *Amer. Fam. Phys.*, **10** (3), 163.

The relatively rapid decline in anticoagulant activity observed during intermittent intravenous administration of heparin dictates that ideally the test of clotting time used for monitoring should be performed three and one-half hours after a dose. A safe, effective level of anticoagulation is thought to be achieved when the clotting time is approximately doubled.

The whole blood activated clotting time (ACT) appears to be a reliable test for monitoring heparin therapy. The method used by the authors for about three years was originally described by Hattersley in 1966 and utilises vacuum blood drawing tubes containing a small amount of diatomaceous earth. Pre-warmed tubes are taken to the bedside in a 37°C heating block. After the first tube is discarded, because of possible tissue thromboplastin contamination, the second tube is allowed to incubate for 1 min then check every 5 sec for clot formation The test has been found rapid, convenient and reproducible, with a co-efficient of variation of 4 percent. There appears to be good correlation with blood heparin levels.

The normal range for the ACT is considered to be 1 min 15 sec to 2 min 15 sec, with the therapeutic range to be maintained at less than twice the baseline ACT. In no case should patients be allowed to develop ACTs of more than 4 min, and, in general, patients are maintained at a therapeutic range of 3 min to 3 min 30 sec, determined three and one-half hours after heparin injection.

Poor correlation with heparin levels has been reported when using the activated partial thromboplastin time. —J.H.

MICROBIOLOGY

Evaluation of Commercially Available Antisera for the Serotyping of *Pseudomonas aeruginosa*. Al-Dujaili, L. and Harris, D. M. (1974), *J. clin. Path.* **27**, 569

Commercially prepared antisera for the serotyping of *Pseudomonas aeruginosa* are now available. The authors compared the ease of use of these antisera and their value in differentiating strains of Pseudomonas with the Pycone typing method of Govan and Gillies (1969). They conclude that for the smaller laboratory a serological method may be more suitable and simpler than Pyocine typing and that for large laboratories a combination of the two methods may give better overall differentiation than either one used alone. — D.G.B.

Resistotyping of *Proteus mirabilis* and a Comparison with other Methods of Typing. Kashbur, I. M., George, R. H. and Aycliffe, G. A. J. (1974), *J. clin. Path.* **27**, 572.

A resistotyping technique using ten substances was compared with the results obtained by serological typing, Protocine typing bacteriophage typing and the Dienz test. The authors found that the resistotyping method gave better differentiation over the 200 strains tested than the other methods used. No connection between resistotype and antibiotic resistance was found. — D.G.B.

Antibiotic Sensitivity Testing, A Modification of the Stokes Method using Rotary Plater. Pearson, C. H. and Whitehead, J. E. M. (1974), *J. Clin Path.*, **27**, 430.

This technical note describes a method of carrying out the Stokes Technique of antibiotic sensitivity testing using a rotary plater. The method has the advantage in that more than the four disks usually used with the stokes method can be set up. — D.G.B.

The Isolation of Anaerobic Bacteria from Wound Swabs. Peach, S. and Hayek, L. (1974), *J. clin. Path.* **27**, 578.

This paper discussed a comparative trial of three methods of isolating anaerobes from clinical material. The methods used were an anaerobic glove box technique using prereduced blood agar, the immediate plating of swabs onto unreduced blood agar and their immediate incubation anaerobically in McIntosh and Fildes jars and laboratory's normal technique of seeding swabs onto blood agar which were then left until sufficient had accumulated to fill an anaerobic jar. Using the anaerobic glove box method anaerobes were isolated from 425 swabs, using the non-prereduced medium with immediate incubation. 135 of the specmens produced anaerobes and using the laboratory's normal technique only 45 produced anaerobes. The startling differences shown appear to be largely due to the use of prereduced media and must make all laboratories carrying out anaerobic bacteriology take a searching look at their techniques. — D.G.B.

A Test System for the Biological Safety Cabinet. Newsom, S. W. B. (1974), *J. clin. Path.* **27**, 585.

The author of this paper quite rightly starts from the thesis that any department which has need for a biological safety cabinet must also be in a position to test its function. To this end he describes a simple method of testing such cabinets using a Bird micronebuliser. This instrument provides a cheap and effect-

ive generator of bacterial containing aerosols and used in conjunction with a slit sampler and anemometer enables effective tests of biological safety cabinets can be carried out. — D.G.B.

Comparative Sensitivity and Resistance of some Strains of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* to Antibacterial Agents. Russel, A. D. and Mills, A. P. (1974), *J. clin. Path.* 27, 463.

The resistance of *Pseudomonas aeruginosa* and *P. stutzeri* to chlorhexidine diacetate, cetrimide, benzalkonium chloride, tobramycin, gentamycin, polymixin B sulphate, ampicillin, carbenicillin, cephaloridine and cephacetrile were examined. The interest in the resistance of *Pseudomonas stutzeri* was due to its frequent occurrence as a contaminant of cosmetic products, particularly in eye make-up. It appears the *Pseudomonas stutzeri* is much more sensitive to all of these substances than is *Pseudomonas aeruginosa*. — D.G.B.

Laboratory Diagnosis of Tropical Diseases with Special Reference to Britain - A Review. Ridley, D. S. (1974), *J. clin. Path.* 27, 425.

A very useful review (which also has considerable application in New Zealand) of the methods useful to a nontropical disease laboratory in the identification of tropical parasitic diseases. — D.G.B.

Sporicidal Activity of Hospital Disinfectants. Kelsey, J. C. MacKinnon, I. H. and Maurer, T. M. (1974). *J. clin. Path.* 27, 632.

The authors of this paper suggest that alcoholic hypochlorite, a freshly prepared solution containing 50 percent methanol and hypochlorite sufficient to provide 2,000 parts per million available chloride with a contact time of 15 minutes is the most effective method of freeing clean heat instruments from spores. Glutaraldehyde also shows some sporicidal activity. The authors re-emphasise the fact that no chemical disinfectant can be relied upon to sterilise. — D.G.B.

Osmotically Stable L-forms of *Haemophilus influenzae* and their Significance in Testing Sensitivity to Penicillin. Roberts. D. E., Ingold, A., Want, S. V. and May, J. R. *J. clin. Path.* (1974), 31, 560.

The authors discuss the difficulties of reading serial antibiotic dilution tests in broth due to the presence of osmotically highly resistant L-forms of *Haemophilus influenzae*. They discuss the significance of this stability in relation to antibiotic sensitivity testing and to the persistence of chronic Haemophilus infections of the respiratory tract. — D.G.B.

A Multiple Inoculation Urea Plate Medium. Fuscoe, F. J. *Med. Lab. Technol.* (1974), 31, 247.

The paper describes a medium for testing bacterial urease production by a multiple inoculation technique. The medium is simple to prepare, cheap and compares favourably with Christensen's medium. This medium contains only half the quantity of urea used in the traditional Christensen's medium. It has been tested against an extensive range of bacteria with satisfactory results. This medium may well be worth trying for those laboratories testing large numbers of strains for urease production daily as it could considerably cheapen the cost of this test. — D.G.B.

Advantage in Combining Sulphamethoxazole with Trimethoprim in an Experimental Model of Urinary Infection. Anderson, J. D., Lacey, R. W., Lewis, E. L. and Sellin, M. A. (1974), *J. clin. Path.* 27, 619.

This is a further paper criticising the combination of sulphamethoxazole and trimethroprim and suggesting that Trimethropim is at least as effective as cotrimoxaole. The authors suggest that laboratory tests give a misleading impression of the two constituents of cotrimoxazole *in vivo*. Further clinical trials should be instituted. — D.G.B.

The One Day Selective Migration Procedure for Detecting Salmonellae in Faeces. Chau, P. Y. and Huang, C. T. (1974), *J. clin. Path.* 27, 405.

A method, by which is claimed a 28 percent increase in the frequency of positive isolations of salmonellae was obtained and a pure growth or almost pure growth of salmonellae can be obtained within 16-24 hours. The method consists of passing the organisms through a U-shaped tube of selective medium, the selective agents being brilliant green and magnesium chloride and the testing of any organisms which migrate through by slide agglutination. The drawback of the technique is that *Salmonella typhi* does not migrate fast enough to be isolated by this method. — D.G.B.

Gram Positive Motile Cluster-forming Cocci as a Cause of Urinary Infection. Virtanen, S. (1974), *J. Clin. Path.* 27, 408.

This paper gives the biochemical characteristics and drug sensitivities of 113 strains of motile gram positive catalase positive cocci isolated from patients with urinary tract infections. These organisms represent 1 percent of the positive findings in the author's laboratory. Reports of the isolation of such organisms are most unusual in other laboratories and the group of motile gram positive cocci is most ill-defined. It may well pay microbiologists to look more closely at coagulase negative "staphylocci" isolated from urinary tract infections. — D.G.B.

Identification of Nonfermentative Gram-negative Rods in Clinical Material. Sandlin, C. (1974) *Amer J. med. Technol* 40 326.

This paper gives a selection of 13 tests for identifying the more common non-fermentative rods to as least genetic and in most cases special level. The tests are mainly simple. Some good points are made, in particular that motility tests on this type of organism should be carried out on plates rather than in stabs where the organisms may not grow and the optimum temperature of incubation for motility is 18°C. The scheme offered seems to provide useful differentiation of these organisms and should be within the capabilities of the smallest microbiology laboratory. — D.G.B.

Evaluation of the *T. pallidum* Haemagglutination (TPHA) Test for Syphilis on "Problem Sera". Kiraly, K. and Prerau, H. (1974). *Acta derm.-vener.*, 54, 303.

From previous evidence and the data obtained from the present study, and under ecological conditions similar to those prevailing in Europe, the TPHA test can be considered a simple and reliable confirmatory reaction for the diagnosis of syphilis to replace the more complicated *Treponema pallidum* immobilisation test and absorbed fluorescent treponemal antibody test. When it is used without discrimination to test all sera, however, its specificity is less satisfactory.

The TPHA test uses tanned sheep RBCs, coated with a sonicate of pathogenic *T. pallidum* harvested from rabbit testes. To eliminate false positive reactions

and cross-reacting antibodies, sera to be examined are treated with a sorbent containing sheep RBC membrane components, normal rabbit testes and *T. reiteri* cell components.

The TPHA test remains positive after treatment even after lipoidal tests become negative. It is therefore suitable for the retrospective verification of syphilis. The TPHA test is thought, however, to be less sensitive than the absorbed fluorescent treponemal antibody test in primary syphilis. This could be explained by a decreased reactivity of the antigen with the IgM type immunoglobulins which form the bulk of antibodies at the onset of syphilitic infection.

The rate of nonspecifically positives has depended upon the geographic location of the study area. The TPHA test with reagents available commercially (Fujizoki Pharmaceutical Co. Ltd.) is highly sensitive with syphilitic problem sera and, in Europe, specific with biologically false positive sera.

The TPHA test is easy to perform and it requires neither highly skilled personnel nor expensive equipment. — J. H.

Vibrio parahaemolyticus **Gastroenteritis from Eating Conchs.** Rhame, F. S. and Werner, S. B. (1974), *West. J. Med.* 121, 66.

V. parahaemolyticus is an enteropathogenic, facultatively anaerobic, Gram-negative rod which prefers alkaline conditions and a salt concentration of 2-4 percent. Unless this organism is specifically sought, it may not be discovered on routine culture. It grows with more difficulty than *V. cholerae* on MacConkey's medium. Neither of these organisms grows on Salmonella-Shigella agar or EMB agar. Both grow well on thiosulphate-citrate-bile salts-sucrose (TCBS) agar, which has a high salt concentration and a pH of 8.6.

In the present case, TCBS agar was used because of the history of recent consumption of an uncooked seafood product. Many cases of gastroenteritis due to *V. parahaemolyticus* undoubtedly go unrecognised because of failure to culture stool specimens on appropriate media. Stable, dehydrated TCBS agar is commercially available.

V. parahaemolyticus was first recognised as a cause of human gastroenteritis in 1950 in Japan. It is now recognised as one of Japan's most common causes of foodborne illness. In the warm summer months it accounts for up to 50 percent of the cases. The organism is widely distributed in the coastal waters of the world, including many areas of the US. It has been found in a variety of marine fish, shellfish, mud, sediment and water samples primarily from offshore locations. Soft tissue infections have occurrred but most disease and all outbreaks have been limited to gastroenteritis caused by contaminated seafood.

Isolates from diarrhoetic stools almost always demonstrate the Kanagawa phenomenon while isolates from the enviroment usually do not. The heat-stable haemolysin responsible for the Kanagawa phenomenon lyses human erythrocytes (Wagutsuma's agar) but not equine erythrocytes.

— J. H.

What's New?

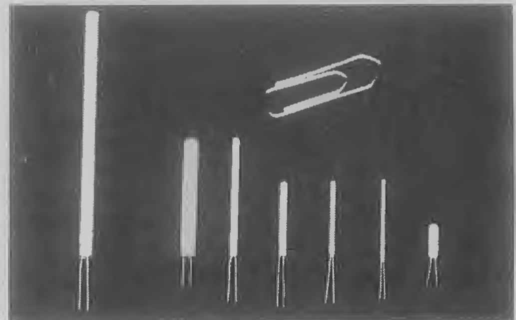
PLATINUM-RESISTANCE TEMPERATURE DETECTORS MADE TO CRITICAL STANDARDS

Matched expansion-coefficients ensure stable, strainfree operation.

Small platinum-resistance temperature detectors suitable for measuring temperature between —200°C and +750°C have been developed by Sensing Devices Ltd., Southport PR9 OBG, England. The three materials of which they are made—platinum wire, high-temperature glass, and a high-alumina ceramic body—have compatible co-efficients of expansion so that the detectors will give consistent readings and remain free from mechanical strain despite repeated thermal shocks. After ten consecutive shocks from 0°C to +600°C or from 0°C to —200°C, for example, resistance at 0°C will not change by more than 0.05 percent.

Several ice-point resistance values between 10 and 400 ohms are available, standard values being 100 or 130 ohms. Resistance/temperature relationships conform to British (BS1904:1964), European (DIN 43760), US and Japanese Specifications. Response times and vibration resistance both conform to BS1904:1964.

The detectors vary in size from 50 mm long × 3.17 mm diameter (the largest) to 6 mm × 2 mm (the shortest) to 15 mm × 0.9 mm (the thinnest). Standard units are available from stock. Detectors can also be supplied in stainless-steel, brass or plastic



housings to purchasers' specification within 2-3 weeks. Their main applications are in textile machinery, chemical and power plant and laboratory and medical equipment, including instruments and autoclaves. An unusual application employs them as miniature high-temperature heaters.

The extruded platinum wire of 0.15, 0.25 or 0.45 mm diameter is specially formulated, and the glass is blended by Sensing Devices themselves to an exact specification. The ceramic bodies are ground to a tolerance of 0.025 mm.

Further information from: Sensing Devices Ltd., 6 Gordon Street, Southport, Lancashire PR9 OBG, England.

Directions for Contributors

These instructions are provided with the object of ensuring uniformity of presentation. Manuscripts should be typed double spaced, on one side only of good quality paper with one inch margins. Carbon copies are not acceptable. Give the author's name with initials if male, or one christian name if female, and the address of the laboratory where the work was carried out. Use capitals only where indicated and do not underline except where italics are required.

In general, papers other than reviews, should consist of a short summary capable of standing alone as an abstract; an Introduction (outlining the problem and the proposed solution); Material and Methods; Results and Discussion.

Illustrations

Illustrations are costly and should be used sparingly. Graphs, line drawings and photographs are all referred to as 'Figures' and should be numbered in the order of their appearance in the text using arabic numerals. Drawings (in indian ink on stout white paper) and photographs, should be about twice the size of the actual reproduction. The position of figures in relation to the text should be noted in the typescript. Legends typed on separate sheets are numbered to correspond with the illustrations. Tables should be typed separately and numbered in roman numerals.

Nomenclature and Units

Scientific names of micro-organisms should conform with Bergey's Manual of Determinative Bacteriology. The first time an organism is mentioned the full generic name should be given and underlined to indicate that it is to be printed in italics. Subsequently it may be abbreviated. Trivial or common names are printed in roman, e.g., staphylococci, and should not be underlined.

To conform with the Systemes Internationale D'Unites or SI units it is recommended that the following prefixes and abbreviations be employed.

Length: m, cm, mm, μm, nm.
Area: m^2, cm^2, mm^2, μm^2.
Volume: litre, ml, μl, nl, pl ('litre' in full avoids confusion with 'l')
Mass: kg, g, mg, μg, ng, pg.
Mass concentrations: kg/litre, g/litre, mg/litre, μg/litre. For the present concentrations per 100 ml also accepted as are daily outputs in urine and faeces.
Molar concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre. (For the present mequiv/litre may also be used.)
Temperature: Express as °C.
Time: s, min, h, d, a. The latter two symbols which stand for day and year respectively are best expressed in full to avoid confusion.
Density: kg/litre (relative density replaces 'specific gravity')
Clearance: litre/s, ml/s (for the present ml/min may also be used).

N.B.:

1. The symbol for a unit is unaltered in the plural and should not be followed by a full stop, e.g., 5 cm not 5 cm. nor 5 cms.

2. No space should be left between the symbol for a prefix and the unit. A space is left between the symbols in derived units,

e.g., ms = millisecond
m s = metre x second

Where ambiguity could arise abbreviations should be written in full.

3. **Numbers.** The decimal is indicated by a full stop. Commas are not used to divide large numbers but a space is left after every third digit.

A zero should precede numbers less than unity. Units which give a number between 0.1 and 1000 should be chosen when possible.

References

References should be listed alphabetically at the end of the article and numbered to correspond with the numbers used in superscript within the text. Citations in the text should give the author's name using *et al.* if more than one author, and the year, thus: Walker *et al.* (1972)[1]. All authors' names should be listed with initials; year of publication in brackets; journal title abbreviated and underlined to indicate italics; volume number in arabic numerals underlined with a wavy line to indicate bold type and the first page number. The reference for abbreviations is the World List of Scientific Periodicals. In general nouns have capitals, adjectives do not and conjunctions are omitted. Authors are referred to previous journals for examples.

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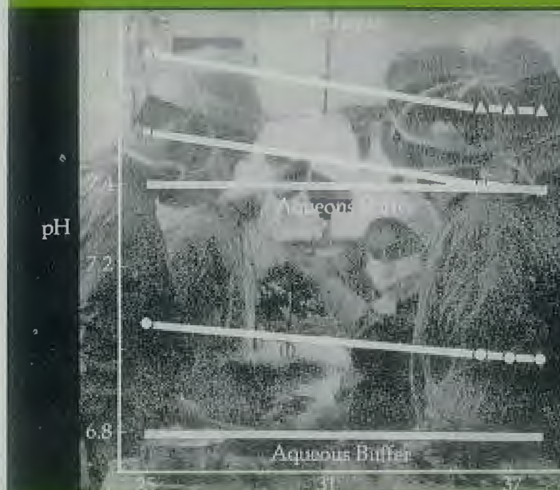

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