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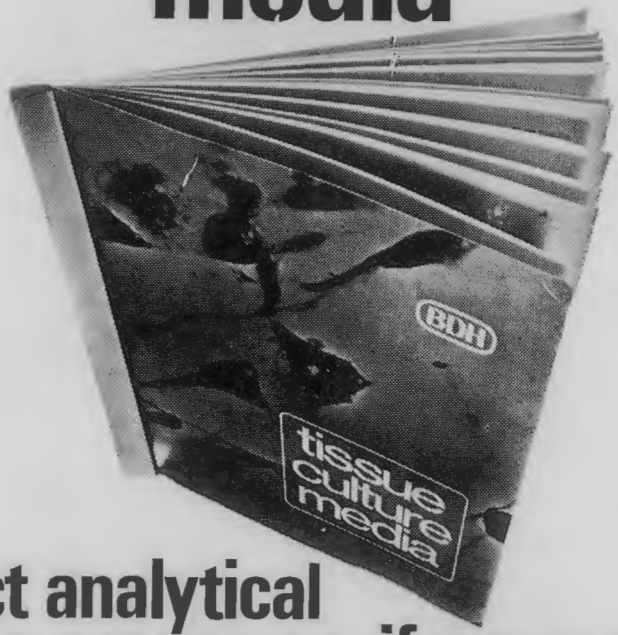

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T. H. Pullar Memorial Address

Education of Medical Laboratory Technologists

Peter B. Herdson, MB, ChB, BMedSc, PhD, FRCPA

Professor of Pathology, University of Auckland School of Medicine

Delivered at the 28th Annual Conference of the New Zealand Institute of Medical Laboratory Technology, Tauranga, August 1972

This is the sixth annual meeting of the New Zealand Institute of Medical Laboratory Technology at which the late Dr Thomas H. Pullar has been remembered in the address established to honour him. Former speakers on this occasion have included Dr Phillip P. Lynch, Dr Fred W. Gunz, Dr Stephen E. Williams, Professor Neil P. Markham, and Dr William L. Kenealy, and I am deeply conscious of the honour you have done me in asking me to join the ranks of these senior New Zealand pathologists in giving this address today.

Each of these former speakers knew Tom Pullar well, and I am only sorry that I did not. His name is of course known to anyone interested in pathology in New Zealand over the past 30-odd years, but it happened that I had not met Dr Pullar before I went overseas in 1961, and he died in 1966. It is a mark of the man that he is so warmly remembered among all pathologists and laboratory technologists who knew him. I have found it interesting and stimulating to talk with a number of people who knew and worked with Dr Pullar, in order to give me some background to this address in his memory.

Each of the pathologists who has given this address in the past has included some background to Dr Pullar's career. It was my pleasure to be present at the 25th Annual Conference of this Institute soon after I returned to Auckland, when Dr Williams spoke of Dr Pullar. As you know, Dr Pullar worked from 1938 for many years at Palmerston North, and then all too briefly here in Tauranga. When I knew that this Institute was holding its 1972 meeting in Tauranga, it occurred to me how fitting it would be if Dr Graham Somerville gave this address, for I knew that Dr Pullar had joined Dr Somerville in practice here in Tauranga before he died. I also knew a little of the circumstances, wherein Dr Somerville had just left for an extended overseas business trip when Dr Pullar died, a circumstance wherein Dr Stephen Williams characteristically provided tremendous support to those left in Tauranga to carry on the work of the laboratory.

And so I was surprised when approached by members of this Institute asking if I would give this address. On inquiry, it transpired that Dr Somerville expected to be overseas at this time, and that one way or another he and his partner, Dr Philip Palmer, had manged to put me in the hot seat.

Of the many contributions that Dr Pullar made to laboratory services here in New Zealand, none is greater than his early recognition of the importance of technologists and their education and status within the laboratory services of New Zealand. And so it seems appropriate for me to spend a little time this morning discussing the question of technical education as it applies to laboratory practice in New Zealand.

You are all well aware of recent developments in the training programmes for medical laboratory technologists. I think it is fair to say that the establishment of the paramedical option of the New Zealand Certificate in Science has been a significant step forward. This year a number of people are expected to graduate from this three-year course. The course is established now in the Technical Institutes at Auckland, Wellington, Christchurch and Dunedin, and very few problems have arisen with regard to laboratory personnel attending courses in these centres. Block courses have been established at the Central Institute of Technology in Petone and in general these have proved satisfactory. There have been some problems with regard to block release from smaller centres, and also some of the people attending these courses have found it a considerable strain because of the intensive brief nature of the block course. However, on balance, the establishment of the Paramedical New Zealand Certificate in Science appears to be a step in the right direction.

With the establishment of this certificate course three years ago, people began to con-

sider the sequence to be followed for those attaining the New Zealand Certificate in Science, which already it was agreed was equivalent to the old Basic Training Certificate. At the moment, holders of either of these qualifications can proceed to the Certificate of Proficiency, requiring two further years' work, and then further in-service work can result in Fellowship of the New Zealand Institute of Medical Laboratory Technology. It is particularly the question of this post-NZCS training programme which at present is so much under discussion.

Mr H. Hutchings of this Institute has been in the forefront of discussions about training programmes, with particular regard to possible inter-relations with Massey University. Right away, then, we get into the question of possible inter-relationships between training programmes in technical institutes and programmes in universities.

Many of you have heard me say before that I believe the whole question of tertiary education in New Zealand is of vital importance to us all. I think we are coming out of the dark ages wherein most of the constituent colleges of the University of New Zealand had far too many first-year or Stage I students, proportionate to their total student enrolment, and where in egalitarian New Zealand it was considered somehow preferable to attend university for a year and fail all subjects, rather than getting on with a sound work and training programme based on a technical institute.

I think now that there is a general awareness of the excellence of the programmes available in technical institutes, and also an awareness of the necessity for some cost:benefit relationship to be applied to universities. I emphasise that I believe the contributions to the community of universities in New Zealand over the years has been and will continue to be immense. But I am perhaps out-of-fashion enough to believe that university education is not something which is a God-given right, but rather something to be worked for.

Putting it in other words, I very much favour the up-grading of other forms of tertiary education; this up-grading must come first by content, with a resultant up-grading in status.

I am convinced that the answers to laboratory technology training do not lie entirely with the universities. I may be treading on a few toes by saying that there is a work-load in the laboratory which must be met, and that no amount of airy-fairy discussion will serve to get that work-load done. I believe the work-load is an honourable one, a satisfying one, and one which, for many people, requires no further justification.

This is not to gainsay that there is a crying need for high-powered personnel in the laboratory. Of course there is, and here again I believe that we are leaving behind another dark age, in that at least we have lain the ghost that someone qualified in medicine automatically will be a tremendous addition to any aspect of the laboratory, whereas someone qualified in laboratory work has little to contribute to clinical medicine. On the contrary, it is obvious that a person highly trained in aspects of, let us say, chemistry, has a very precious usefulness in the chemistry laboratory, which is not equalled simply by gaining a qualification in medicine. On the other hand, it is equally obvious that qualification and experience in medicine is a tremendous help in interpreting the results of the laboratory with regard to particular patients.

A group of us in Auckland, including several senior members of this Institute and Dr Stephen Williams, have wondered about the advisability of seeking to establish a Diploma in Medical Laboratory Technology in the University of Auckland. The type of thing that we have discussed would involve, let us say, a three-year course, the requirements for the first year of which could be met by high achievement in the New Zealand Certificate in Science Paramedical Course, with the succeeding two years being a university adaptation of the present course for the Certificate of Proficiency. Subjects to be covered in such a Diploma course may include aspects of general pathology, behavioural science, principles of medical practice, clinical pathology, principles of public health, and laboratory administration. The course may be designed as a two-year one, occupying half of each day.

This suggestion raises a number of points, some of which already we are considering in Auckland.

Firstly, the University of Auckland is restricted in the number of students it can take at present. The roll is limited to approximately 10,000 students, and accordingly the institution

of a new course is looked at in the context of the effect it will have on student numbers. I do not think this is a serious concern with regard to our proposed Diploma course, because on the one hand the numbers of people likely to be taking the course will be small, and on the other, it is envisaged that the entire course would be undertaken on the Medical School site, which at the moment is not subject to the embargo on increased student numbers which applies to the main university site centred on Princes Street.

Secondly, we must ensure that there is adequate space for such a proposed course. We expect to have Government approval to proceed with extensions to the present Medical School, in order approximately to double the intake of medical students on the site, and these extensions will include enlargement of facilities for pathology. In turn, I would hope that we will be able to plan the timing of the proposed Diploma course in such a way that the enlarged facilities for pathology will cope not only with the increased number of medical students and graduate students in pathology, but also with those taking the Diploma course.

Thirdly, we come to the question of staff. Here it is vital that we have approval from the University Grants Committee to appoint the necessary additional staff to deal with the proposed Diploma course. At this stage, we cannot be sure as to the precise requirements, but it would seem to me that we will require the services of the equivalent of one full-time senior lecturer for this Diploma course. In practice, I would imagine that we will be needing the services of several part-time members of staff, some of whom clearly we would hope would be senior members of this Institute.

Fourthly, we have not clarified the relationship between the proposed Diploma course and the present Certificate of Proficiency courses. At the moment, it would seem that we will need both, in that the curricula of the two are complementary.

Another matter of broad importance concerns the inter-relationship between the training programmes available to members of this Institute, and those undertaken by students at the university leading to the degrees of BSc. MSc and PhD. The question of cross-crediting between courses leading to the New Zealand Certificate in Science (Para-medical) and Certificate of Proficiency with the University Faculty of Science is at present under consideration. In Auckland, we have the precedents of cross-crediting occurring in Engineering and in Chemistry between the New Zealand Certificate in Science and the relevant Bachelor's courses. In general, those who have passed the New Zealand Certificate in Science with high academic achievement may be credited from one-third up to one-half of the course requirements for the Bachelor's degree. This works out that if the BSc requires three full-time years at university a holder of the New Zealand Certificate of Science with high passes in relevant subjects may be credited one of the three years towards the BSc and similarly in Engineering, two of the four years towards the BE. Whilst I do not see any great problems in the establishment of this cross-crediting, quite frankly I think it will be inappropriate in all but the most unusual instance. In other words, I believe that there will be very few circumstances in which a person will enter the training programme in Medical Laboratory Technology, and proceed to gain the New Zealand Certificate in Science (Para-medical), and either then, or after a further two years gaining the Certificate of Proficiency, wish to commence a course leading to a BSc which will require the equivalent of a further two years' full-time work. In my opinion, it is vital that we arrive at a first-rate training programme and career structure in Medical Laboratory Technology. And in this context, I believe that cross-crediting as just outlined will be applicable only to a very few, and in fact in most instances will be quite inappropriate. I say this in the context that a BSc these days is in fact of less commercial value than the relevant qualifications in Medical Laboratory Technology already available.

In all of these discussions, there is another aspect to be considered, and again I may tread on a few toes; it is the fact that a large number of personnel coming into Laboratory Technological Training Programmes are not working in laboratories five years after they start. With the present arrangements, a large proportion of trainees in the laboratory are girls and it would be a sad day for New Zealand

if the vast majority of young women commencing training in the laboratory gave away all thoughts of marriage for the betterment of pathology. One immediate and obvious question is what can we do about attracting more men to laboratory technology services? This is a complex question, which I won't attempt to answer here, save to say that I do not think the answer lies merely in a salary structure. In fact, present salaries in Medical Laboratory Technology are pretty reasonable. No, I believe the answer is related to the whole structure of the training programme and career opportunities in Medical Laboratory Technology.

There are pros and cons in inservice training programmes and full-time degree courses, as they relate to any aspect of life. I have never regretted doing a four-year inservice training programme prior to attending university, for I believe that it gave me an insight into what could be gained from university which I would not have had otherwise. But just as full-time attendance at university cannot provide experience in service laboratories, so full-time or nearly so attendance at university is necessary for a university course. In other words, I believe that we should seek more to provide mechanisms whereby persons interested in laboratory technology work as a lifetime career can attain relevant qualifications, and have a career structure which recognises their worth, than get into a series of half-baked quasi-professional courses. I have the utmost respect for the training programmes of the Technical Institutes, and I believe that the options for the vast majority entering laboratory technology are already with us. In Auckland, the School of Laboratory Technology receives something like two qualified applicants for every post each year, in a climate where attendance at university to do Stage I subjects for BSc is possible for most people holding a University Entrance qualification. Obviously, there are a number of other factors, probably the most important being finance. It seems to me that we should be re-emphasising the honourable rewards for those who work in laboratories and seek to gain their New Zealand Certificate in Science whilst being paid for the service aspects of their work. Many members of this Institute over the last two or three years have said that this qualification should be recognised as being the basic one for work in the laboratory, and that it should be fair and reasonable for a significant proportion of those attaining this certificate to feel that that is their qualification to proceed with their work. For the smaller number who wish further training, it is probable that the Certificate of Proficiency will continue to provide a reasonable avenue of further training.

And then, a course such as that briefly outlined this morning leading to a Diploma in Medical Laboratory Practice would provide a worthwhile experience for those who have the desire and stamina. With the establishment of cross-crediting allowing those who wish to pursue a 'standard' university course, eventually leading to BSc, MSc or PhD, it would seem that we have a good framework to build on over the next decade or so.

Well, I've briefly covered a few aspects of the vast subject of 'Education of Medical Laboratory Technologists'. These comments arise out of many discussions with a number of people, and I am very grateful for the helpful comments of many on this subject.

Enough for now—but in closing, I must say that with the problems of education for Medical Laboratory Technologists very much on my mind last evening, I was delighted to be informed that your Executive is considering setting up a special committee to make recommendations on the 'Training of Pathologists'. This appeals to me, and I am sure would have appealed to Dr Pullar.

Antibiotic Sensitivities of Staphylococci

Susan M. Harding, BSc, ANZIMLT, and Elizabeth Cooper
National Health Institute, Wellington

Received for publication, May 1972

Introduction

There have recently been a number of reports of outbreaks of infection due to multiple resistant staphylococci[2] and reports of increasing resistance to certain antibiotics such as neomycin and methicillin [1, 6, 7, 8]. The purpose of this study was to determine whether multiple resistance is becoming common in New Zealand, and to relate resistance to certain phage types.

Materials

Three hundred and eighty-three (383) coagulase positive staphylococci from hospital patients were tested for sensitivity to penicillin, erythromycin, neomycin and tetracycline. Any strains that were resistant to penicillin were also tested for sensitivity to methicillin. The cultures were selected to give a random sampling from different hospitals. Three hundred (300) of the staphylococci were miscellaneous phage types, taken from cultures received for phage typing between April and July 1969 and including only one from each patient.

The remaining 83 included 15 staphylococci each of phage types 52/52A/80/81, 84/85, 75/77/84/85, 47/53/54/75/85; 17 of phage type 80/81; and 6 of phage type 88 (from 1969 and 1970). These cultures were selected separately as they were known to be resistant to several antibiotics or of significant epidemiology, either in New Zealand or other countries. A Table (1) is included to show the incidence of predominant strains phage typed at the National Health Institute during 1969. These cultures were received mainly from hospitals in the North Island and do not cover the whole of New Zealand.

Strains of 75/77/84/85 and 47/53/54/75/85 have caused minor outbreaks of infection in several hospitals in this country. Strain 84/85 (originally B5/77Ad) has occurred in a number of outbreaks in other countries [4, 5, 9], but has not been commonly found in New Zealand. The strains 80/81 and 52/52A/80/81 were found in large numbers in New Zealand a few years ago, but are now much less common although still of importance. Strains of phage type 88 were included as this strain has been found to be methicillin resistant in Britain (Report of the International Sub-committee on Phage Typing of Staphylococci, 1966-70).

Table 1: Coagulase Positive Staphylococci Typed In 1969

Number of Staphylococci in Each Group

Group	Number	Percentage
Group I	582	20.4
Group II	333	11.6
Group III	922	32.2
Miscellaneous and Mixed	326	11.4
Untypable	699	24.4
Total	2,862	100.0

Number of Staphylococci of Specific Types and Percentage of Total Staphylococci

Type	Number	Percentage
80/81	143	5.0
52/52A/80/81	119	4.2
3c/55/71	147	5.1
71	50	1.7
84/85	22	0.8
75/77/84/85	144	5.0
47/53/54/75/85	139*	4.9
53	34	1.2
187	17	0.6
42D	9	0.3

* (122 of these were from an outbreak in one hospital)

Method

Plate dilution and disc sensitivity methods were used for all cultures. Five-hour broth cultures from a single colony inoculation were used to give approximately uniform growth. The media used were Nutrient Broth (Difco) and Trypticase Soy Agar (B.B.L.). The Oxford Staphylococcus (N.C.T.C. 6571) was used as the control on all plates. The plates were incubated overnight at 37°C except for those with methicillin discs which were incubated for 18 hours at 30°C[3].

Plate Dilution Method

The correct dilution of antibiotic was mixed with the required amount of agar to give a final volume of 20 mls, and then poured into a plate giving a depth of 5 mm. After drying the plates were inoculated with 24 test organisms and the Oxford control by means of a multi-point inoculator.

Antibiotics were used at the following concentrations:

Penicillin —0.1, 1, 10, 100, 250, 500 units/ml.

Erythromycin—0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 50 mcg/ml.

Neomycin —0.5, 1, 2.5, 5, 10, 25, 50, 75, 100 mcg/ml.

Tetracycline —0.5, 1, 2.5, 5, 10, 25, 50, 75 100 mcg/ml.

Methicillin —1, 5, 10 mcg/ml.

Disc Sensitivity Method

The agar plates each contained 20 mls of agar which gave a depth of approximately 5mm. For each plate a test strain was swabbed on to a centre panel, with the control strain on either side. The discs were then placed on the plates so that they were half on the test strain and half on the control strain (The Association of Clinical Pathologists, Broadsheet No. 55). After incubation the diameters of the inhibition zones were measured.

Disc concentrations were:

Penicillin	— 10 units
Erythromycin	— 15 mcgs
Neomycin	— 30 mcgs
Tetracycline	— 30 mcgs
Methicillin	— 10 mcgs.

Results and Discussion

The results of disc sensitivities are summarised in Table 2. Strains were recorded as resistant if the zone diameter was less than half that of the control.

Table 2: Disc Sensitivities

		Number of Strains Resistant				
	Total Number	Penicillin	Erythromycin	Neomycin	Tetracycline	Number Sensitive to all 4
All Cultures	383	225	42	20	77	128
		58.7 %	11.0 %	5.2 %	20.1 %	33.4 %

		Number of Strains Resistant				
Miscellaneous Staphs.	Total Number	Penicillin	Erythromycin	Neomycin	Tetracycline	Number Sensitive to all 4
Group I	46	25	2	0	2	19
Group II	62	41	1	0	5	18
Group III	86	56	3	1	3	28
Miscellaneous	58	27	2	0	6	27
Untypable	48	20	0	0	0	28
Total	300	169	8	1	16	120
		56.3 %	2.7 %	0.3 %	5.3 %	40.0 %

		Number of Strains Resistant				
Specific Phage Types	Total Number	Penicillin	Erythromycin	Neomycin	Tetracycline	Number Sensitive to all 4
47/53/54/75/85	15	12	14	0	15	0
75/77/84/85	15	13	1	0	13	0
84/85	15	6	15	14	15	0
80/81	17	13	0	0	9	2
52/52A/80/81	15	8	0	0	4	6
88	6	4	4	5	5	0
Total	83	56	34	19	61	8
		67.5 %	40.1 %	22.9 %	73.5 %	9.6 %

Penicillinase production was observed (Broadsheet No. 55) but all penicillinase producers had zones of inhibition less than half that of the control so were recorded as resistant regardless of penicillinase production.

All strains were sensitive to methicillin. Penicillin resistance appears to be fairly evenly distributed throughout all the groups, nearly 60 percent being resistant. It is interesting to note that only six out of 15 (40 percent) 84/85 strains were penicillin resistant, whereas these strains were resistant to erythromycin, tetracycline and neomycin.

Only 42 (11 percent) cultures were resistant to erythromycin but of these 29 fell into two types (47/53/54/75/85, and 84/85). Seventy-seven (77) cultures (20.1 percent) were resistant to tetracycline and 43 of these fell into three types (47/53/54/75/85, 75/77/84/85, and 84/85). Of the 20 neomycin-resistant strains, 14 were 84/85. 33.4 percent of the cultures were sensitive to all four antibiotics, but none of the strains 47/53/54/75/85, 75/77/84/85, and 84/85, were completely sensitive, 40 out of 45 being resistant to two or more antibiotics. 48.8 percent of the cultures were resistant to one antibiotic, but apart from the specific types only 4.3 percent cultures were resistant to more than one antibiotic (Table 3).

These results are very similar to those obtained in 1967 (unpublished) when 84/85, 47/53/54/75/85, and 75/77/84/85 were generally found to be resistant to several antibiotics, whereas 80/81 and 52/52A/80/81 are usually only resistant to one or two antibiotics and most other types are resistant to penicillin only or completely sensitive.

Correlation of the disc and plate sensitivities was fairly good with the exception of erythromycin, where some strains which appeared resistant by the disc method were only resistant to low concentrations of the antibiotic by the plate method. It was noted that 80/81 and 52/52A/80/81 strains were resistant to greater quantities of penicillin (500 or more units) than strains 47/53/54/75/85 and 75/77/84/85, which were resistant to 250 units or less. These two strains tend, however, to be resistant to more antibiotics than 80/81 and 52/52A/80/81.

It is hoped that a more detailed comparison between disc and plate sensitivities will be the subject of another communication but it would seem from our results that disc tests are satisfactory in most cases for determining resistance, although no accurate indication of the degree of resistance can be obtained from the size of the zone.

Conclusion

Apart from the specific phage types there are very few strains resistant to more than one antibiotic.

Neomycin resistance is confined to two strains, and no methicillin-resistant strains have been found. It would appear that multiple resistance in New Zealand is associated with a few specific strains, and these strains do not occur in large numbers.

Table 3: Disc Sensitivities
Resistance to Number of Antibiotics

	Total Number	Resis. to 1	Resis. to 2	Resis. to 3	Resis. to 4	Resis. to NONE
All Cultures	383	187	36	23	9	128
		48.8 %	9.4 %	6.0 %	2.4 %	33.4 %

Miscellaneous Staphs.	Total Number	Resis. to 1	Resis. to 2	Resis. to 3	Resis. to 4	Resis. to NONE
Group I	46	25	2	0	0	19
Group II	62	42	1	1	0	18
Group III	86	53	5	0	0	28
Miscellaneous	58	27	4	0	0	27
Untypable	48	20	0	0	0	28
Total	300	167	12	1	0	120
		55.7 %	4.0 %	0.3 %		40.0 %

Specific Phage Types	Total Number	Resis. to 1	Resis. to 2	Resis. to 3	Resis. to 4	Resis. to NONE
47/53/54/75/85	15	1	2	12	0	0
75/77/84/85	15	4	10	1	0	0
84/85	15	0	1	8	6	0
80/81	17	8	7	0	0	2
52/52A/80/81	15	6	3	0	0	6
88	6	1	1	1	3	0
Total	83	20	24	22	9	8

Acknowledgments

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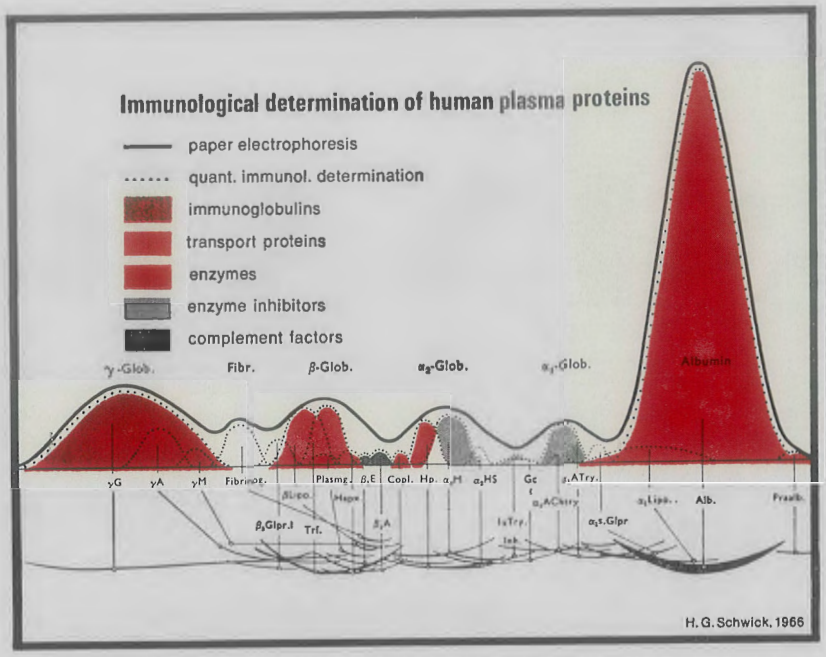

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Anti-P_1 and Hydatid Cyst Fluid

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Summary

A simple and inexpensive method of confirming the identification of anti-P_1 using hydatid cyst fluid is described. This material can be easily obtained. The significance of anti-P_1 and the techniques used in its identification are discussed.

Introduction

Anti-P_1 is a common agglutinin accounting for 34.5 percent of all antibodies and 76.8 percent of the naturally-occurring antibodies identified in the Palmerston North Public Hospital laboratory in a recent twelve-month period. The frequency with which anti-P_1 is detected varies between laboratories. Its reactivity is greatly influenced by the temperature at which the test is carried out and by the sensitivity of the technique used. Henningsen (1949)[2] reported that anti-P (anti-P_1) was present in two-thirds of P negative (P_2) people, and in almost 90 percent of P_2 pregnant women, although there seemed to be no iso-immunisation during pregnancy. The antibody was usually weak and reacted only at low temperatures.

Anti-P_1 is a common cause of cross-matching difficulties and although its clinical significance may often be in doubt the laboratory technologist is not in a position to release blood for transfusion when he knows it to be incompatible *in vitro*. Where incompatibility is demonstrated in the room temperature, saline-agglutination test the practice of re-cross-matching the blood using a 30°C, saline-agglutination technique is employed by some workers. If there is no reaction in this test the blood is issued and it is assumed that no harm will result.

Very few anti-P_1 agglutinins react at 30°C. However, this practice has some disadvantages and its use should not be encouraged. It tends to negate the usefulness of the room temperature saline cross-match. One must always consider it good policy to identify an antibody and to transfuse blood compatible by the routine *in vitro* tests.

In laboratories using an anti-human globulin reagent containing standardised anti-complement and anti-immunoglobulin M components many naturally occurring antibodies will be detected in the indirect Coombs' test phase of the cross-match, so that the cross-match may still be incompatible.

To identify the antibody it must be tested against a panel of cells. The panel should consist of at least ten carefully chosen cells, including at least two cells with strong P_1 antigens and at least three P_1 negative cells. The strong P_1 antigens will provide greatest sensitivity at the selected temperature. The relative strengths of the P_1 antigens of the panel of cells should be carefully assessed and recorded. This is best done after several examples of anti-P_1 have been identified.

Material and Methods

The technique used in this laboratory is to add one volume of a two percent washed-cell suspension to one or two volumes of the patient's serum in disposable polystyrene 5 mm bore test tubes. (Obtainable from Henleys Medical Supplies Ltd., Alexandra Works,

Clarendon Road, London, N.8. Ref: GL/1. New Zealand agents: Surgical Appliances Ltd., 311-315 Great North Road, Auckland 2.)

Without mixing, the cells are allowed to settle at room temperature for at least one hour. The tests are read microscopically after gentle resuspension using a Pasteur pipette.

P_1 typing of the patient's cells and prospective donors will help confirm the identity of the antibody and the compatibility of the donors. However, sera suitable for typing are difficult to obtain. Anti-P_1 of sufficient titre and avidity is uncommon in the population. Satisfactory sera can be produced in animals, but the facilities required are not available to all. (Levine *et al.*, 1958[6]; Prokop and Oesterle, 1958[7]; Levine and Celano, 1959[5]; Kerde *et al.*, 1960[4]; Watkins and Morgan, 1964[10]). Many Laboratories must purchase the reagent commercially and in view of the large number of anti-P_1 agglutinins detected the expense can be considerable.

An alternative method of confirmation of the identity of the antibody is to use a neutralisation test with hydatid cyst fluid (HCF). Cameron and Staveley (1957)[1] investigated HCF from sheep livers and found that provided the cysts contained active scolices the fluid contained P_1 substance. Saline dilutions of the fluid were used to neutralise a potent anti-P_1. In eighteen samples of HCF the results showed complete neutralisation of the anti-P_1 in dilutions ranging from 16-256.

The HCF is convenient to use by adding one volume to one volume of serum in a disposable polystyrene 5 mm bore test tube.

These must be mixed together and left for neutralisation to occur. Ten minutes is an arbitrary, but satisfactory time. Add one volume of strongly P_1 positive cells and incubate the test for one hour at room temperature. A control substituting 0.9 percent sodium chloride for HCF should be tested in parallel to guard against the possible effects of dilution when the antibody is very weak. Under these circumstances it is desirable to use two volumes of serum in the test.

It appears not to be essential to dilute the HCF, but if a titration in 0.9 percent sodium chloride is performed and a concentration twice that which will just completely inhibit a strong anti-P_1 is used, this is a satisfactory economy. The test can be performed easily as a routine addition to the room-temperature saline panel identification.

HCF has a further use in the identification of mixtures of naturally-occurring antibodies, where it can be used to neutralise anti-P_1, thus enabling other antibodies to be easily identified using a panel containing P_1 positive cells. In this case it is preferable to use the HCF undiluted and to add it to the bulk test serum. This overcomes any effects of dilution and enables the neutralisation to be performed in one operation.

Add twice that amount estimated (or proven by test) to be required to neutralise a strong anti-P_1. A recent example of a mixture of anti-P_1 and anti-Le^{a+b} was identified without difficulty using this technique.

In addition to neutralising anti-P_1, HCF also neutralises anti-P^k, however this is of little significance in its routine use as anti-P^k has been reported only as a component of anti-PP_1P^k in rare p people. In the uncommon event of this antibody being found it would show itself by reacting with all common cells, and although HCF will neutralise the anti-P_1 and anti-P^k components, the anti-P will still react with all common cells (Race and Sanger)[8]. Of more practical importance is the finding that HCF partially or wholly inhibits some anti-I (Tippet *et al.*, 1960[9]; Issett, 1967[3]). Again the fact that the antibody is not anti-P_1 should be readily recognisable from the pattern of reactions of the antibody. Anti-I reacts with all common adult cells and gives negative or weak reactions with cord cells.

Hydatid cyst fluid is obtainable from abattoirs or killing and freezing companies and should be clarified by centrifugation before being stored at —20°C. Its use as part of the routine room-temperature agglutinin identification technique provides valuable information for little effort or cost.

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Experiences with a Spectrophotometric Method for the Estimation of Serum Bilirubin in Infants

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Introduction

There are a large number of methods available for estimating bilirubin with varying advantages and disadvantages. An attempt has been made to find a simple reproducible technique for estimating serum bilirubin in infants. The method in use in this hospital for many years was that of Lathe and Ruthven (1957)[5]. This method has not been entirely satisfactory for a number of reasons. The high dilution employed in the method gives low absorbances and this limits the precision of the estimation. There are also problems with the reagents. Sulphanilic acid is difficult to prepare. If heating or vigorous mixing are used to aid solution, the reagent gives low results and this is also observed as the reagent ages. The quality of the methanol used is important as some batches have caused high results in this laboratory. A newly opened bottle of methanol gives more rapid colour development than one which has been used repeatedly. This is due to absorption of moisture from the atmosphere. It has been clearly demonstrated by White *et al.* (1958)[11] that haemolysed sera give low results. The method is sensitive to pH changes; between pH 1.8 and 2.4 the wavelength giving maximum absorption changes from 530 to 560 nm. In general the method is considered to be time consuming. It involves four pipettings which contribute to the overall error and the diazo reagent has to be prepared freshly for each batch of tests.

Modifications of diazo methods which claim to overcome many of the problems inherent in the diazo reaction have been proposed. One variation has been that of Jendrassik and Grof (1938)[4], who measured azobilirubin as its blue form in strong alkali. This method was not investigated as it was felt that the procedure was more complicated than those methods which measure bilirubin directly by its own absorption peak. This paper is a critical study of one such method; the spectrophotometric method of White[11].

Materials and Method

Phosphate buffer 0.1 M, pH 7.4 $\pm$ 0.05.

Serum (0.05 ml) is pipetted into phosphate buffer (3.0 ml) and mixed well. The absorbance is determined at 455 nm and 580 nm. The bilirubin concentration in mg/100 ml is calculated from the expression (A455-A580) $\times$ factor.

The haemoglobin correction was checked by obtaining a spectrum of haemoglobin on a Beckman DB spectrophotometer. The results were checked on the Unicam SP 600 spectrophotometer by determining the absorbance of two oxyhaemoglobin solutions at 455, 575 and 580 nm.

The linearity of the method was tested by preparing dilutions of Dade Bilirubin Control Serum (Dade Division, American Hospital Supply Corporation) corresponding to bilirubin concentrations up to 80 mg/100 ml.

The method was standardised by using bilirubin of very high purity (National Bureau of Standards, Washington) dissolved in 0.1 M sodium carbonate solution and diluted with an 'acceptable serum diluent' according to the recommendations of a committee on a uniform bilirubin standard[8].

To check the precision of the method a series of tests was carried out by several technologists on a pooled serum.

A comparison of the spectrophotometric method with the method of Lathe and Ruth-

ven[5] was carried out. One hundred samples were analysed by both methods.

Results

The spectrum of haemoglobin is shown in Figure 1.

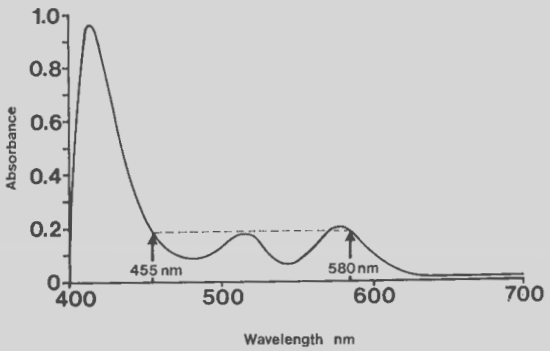

FIG. 1.—Absorption Spectrum of Oxyhaemoglobin.

It can be seen that the absorbance of oxyhaemoglobin is the same at 455 and 580 nm suggesting that the latter wavelength provides a better correction for haemolysis than 575 nm. The readings on the Unican confirm this (Table 1).

TABLE 1.—Absorbence of two arbitrary dilutions of haemolysed washed red cells at 455, 575 and 580 nm.

A455	A575	A580
0.820	0.980	0.820
0.200	0.240	0.204

As the absorbance of bilirubin is negligible at 575 and 580 nm it was decided that 580 nm provided an effective correction for haemolysis.

The standardisation on two occasions gave factors of 72.9 and 73.4. Using a 1 in 60 dilution this corresponds to molar absorbances in phosphate buffer pH 7.4 of 48,000 and 49,200.

The method obeys Beers law up to at least 80 mg/100 ml as is shown in Figure 2.

The method was found to have a standard deviation of ± 0.3 mg/100 ml at a level of 20 mg/100 ml.

The results of the comparison between the two methods is shown in Figure 3.

The regression line for the comparison is $Y = 0.9\ X + 1.34 \pm 0.94$, where Y represents to the bilirubin level estimated by the spectrophotometric method and X represents

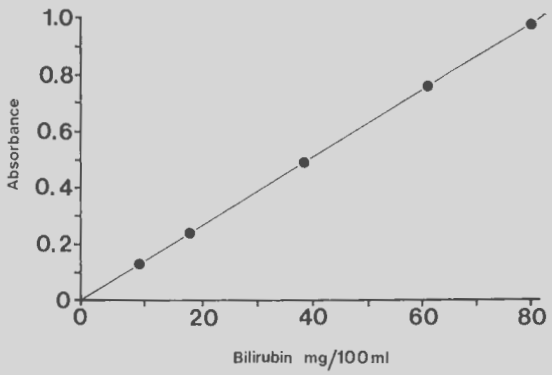

FIG. 2.—Calibration of Bilirubin at 1 in 60 Dilution.

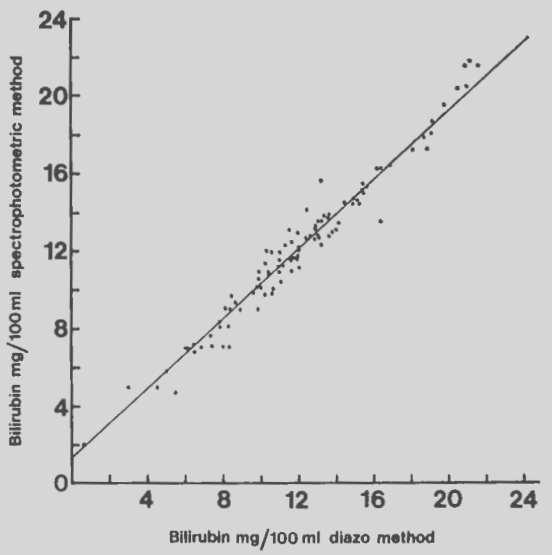

FIG. 3. Comparison of 100 Results by Two Methods.

level by diazo method. The regression line shows that the comparison is poor at low bilirubin levels but very good at high levels.

Discussion

Spectrophotometric methods for the estimation of serum bilirubin are becoming more widely used, and have been recommended as being more suitable for use in neonates than diazo methods (Teitz, 1970[10]). Most spectrophotometric methods use one reading at the bilirubin peak, 450-460 nm and correct for haemolysis either at the Soret peak (415 nm) or in the 540-580 region. White[11] claims that

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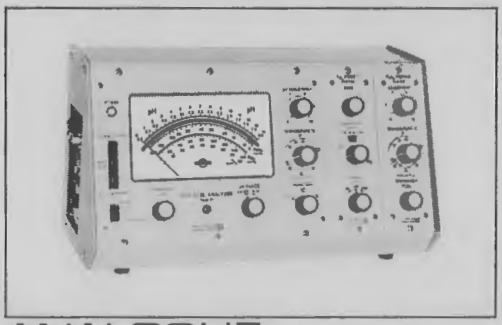

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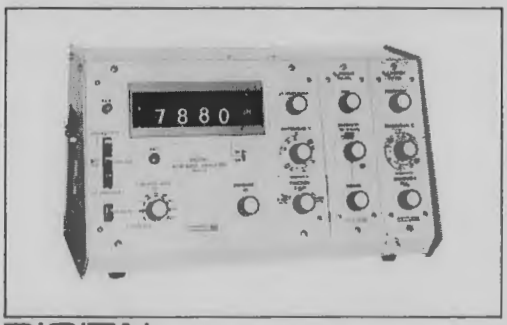

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the Soret peak gives an unreliable correction due to spectral changes in oxyhaemoglobin at this wavelength. By selecting a wavelength where the absorbance of haemoglobin is the same as at the bilirubin peak (as is done in the present method) the calculation is simplified.

The absorbance of turbid solutions increases at shorter wavelengths. This method therefore does not correct for turbidity. Such spectral corrections have been worked out by Fog (1952)[1], but they greatly increase the complexity of the calculation. In our experience it is rare for infants to have turbid sera and when this does occur the sample can be recollected, so it was decided to ignore this correction and to reject samples which showed turbidity.

The spectrum of bilirubin varies with pH (Martin, 1949[6]; With, 1968[12]). This is due to liberation of bilirubin from its albumin complex at acid pH levels. This free bilirubin has an absorbance maximum at 430-440 nm and thus a lower absorbance at 455 nm. The variation in absorbances within the pH range 7.2-7.6 was shown in our laboratory to be almost undetectable but definite though slight variations occurred outside this pH range. It is therefore best to dilute serum in buffer rather than saline. Small errors in the preparation of the buffer would be of little consequence.

The spectrum of conjugated bilirubin differs from that of unconjugated bilirubin (Fog *et al.*, 1967[2]), but as they are so nearly isobestic at 455 nm this does not affect the results.

The calculation given by White[11] gives a factor of 84 when allowance for the 1 in 60 dilution used in this laboratory is made. Use of this factor gave results widely at variance with control sera. This is due to an error in the original paper of White[11], where the absorbance coefficient of haemoglobin at 575 nm has been substituted for that of bilirubin. The reported absorbance coefficients of bilirubin vary so widely in the literature that it is felt that a reliable approach from this coefficient is not possible. Initial attempts to standardise the method from artificially prepared bilirubin were not successful so the method was tentatively standardised against Dade Bilirubin Control (Dade Division, American Hospital Supply Corporation). While on this basis the comparison of the spectrophotometric method with the Lathe and Ruthven method was carried out. The latter method was also standardised on the same basis to give comparable sensitivity. Recently bilirubin has been obtained from the National Bureau of Standards which is claimed to be of high purity. This material is supplied with a certificate of analysis which describes the purity testing procedures in detail. The results obtained with this preparation gave factors which agreed more closely with factors given by quality control sera. Using the factor obtained from this standard, average values for Dade Bilirubin Control serum are 103 percent of their stated value and for Versatol Pediatric (General Diagnostics Division, Warner Chilcott), average 95 percent of their stated value. This is in approximate agreement with the findings of Shelong (1965)[9], who found that control sera from Dade gave 98-101 percent of their stated value while those from Warner Chilcott ranged from 87-92 percent. It is thought that this may be due to variation in the purity of the bilirubin preparations used. The initial unsuccessful attempt at standardisation used bilirubin 'standard reagent grade' (Schwartz Mann). If the bilirubin from the National Bureau of Standards is accepted as 100 percent pure, data obtained in this laboratory would imply a purity of only 91 percent for the commercial bilirubin, which would account for the unsuccessful standardisation.

It would be expected that a method involving one dilution and two absorbance measurements would show a high degree of precision and this is shown in the low standard deviation obtained. It is stressed that for precise results meticulous attention must be paid to the details of pipetting. It is important that cuvettes should be matched. A narrow band pass spectrophotometer must be used although small errors in setting the wavelength are of little consequence.

The accuracy of the method is much more difficult to assess. The method is not specific since any substance with similar absorption characteristics to bilirubin will be measured. One potential interference considered was carotene. The reported normal values of carotene in infants are up to 50 μg/100 ml which would cause negligible interference[10, 3]. The diazo reaction is known to be specific for bilirubin so it seems reasonable to assume from the good agreement shown between the two methods

in Figure 3, that the spectrophotometric method is accurate.

Both accuracy and precision are decreased at low bilirubin levels and are quite poor in the normal adult range. This is of little consequence in infants since only elevated bilirubin levels are of clinical interest but indicates that the method is not suited to bilirubin estimation on samples from adults or on cord blood specimens. The method of Powell (1944)[7], is used for these but the method of Jendrassik and Grof is under investigation in this regard. The use of two bilirubin methods is quite justified since it is felt that none of the diazo methods used for bilirubin estimation on micro samples is suited to the measurement of low bilirubin levels due to the high dilution employed. This view is also expressed by With[12b].

Conclusion

A spectrophotometric method for the estimation of serum bilirubin in infants has been considered. The method has been found to be quick, convenient, accurate and precise. The method is ideally suited to mechanisation. The lack of precision in the diazo method caused fluctuations in the results of serial bilirubins which were not acceptable clinically. These fluctuations have been greatly reduced since the introduction of the new method. The method has been in routine use for six months and is continuing to function satisfactorily.

Acknowledgments

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The Micro TPHA; A Comparison with other Serological Tests for Syphilis

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Summary

The Micro TPHA of Logan and Cox (1970)[8] with some modifications is compared to the FTA-ABS and other serological tests for syphilis. A total of 220 sera were studied and the value of the Micro TPHA procedure for differentiating problematical sera from true treponemal infections is discussed. A significant proportion of treponemal infections were reactive only by the FTA-ABS and TPHA procedures in this series.

Introduction

The diagnosis of syphilis depends upon the correlation of all available historical and clinical data with information provided by the various serological tests. *T. pallidum* infection leads to the production of multiple antibodies which are of two basic types; (1) non-specific tissue antibodies (Reagin) and (2) specific anti-treponemal antibodies.

Reagin tests performed in most laboratories are of two types, flocculation and complement

fixation methods and in this country VDRL and Wasserman tests predominate.

The many standard tests designed to detect the presence or absence of Reagin use cardiolipin-lecithin extracts as their source of antigen. These antigens are, however, found in a number of normal tissues, so it is not surprising that these tests are sometimes falsely positive. The disorders associated with these false reactions have been extensively documented[3, 10]. Transient reactions are not infrequently found during pregnancy and following viral, bacterial, plasmodial and spirochaetal infections. Severe blood loss in susceptible persons can stimulate the production of excess Reagin[5]. Chronic biologically false positive (BFP) results associated particularly with autoimmune disease, lepromatous leprosy and brucellosis cause major diagnostic problems. Old age and narcotic addiction are interesting but poorly understood causes.

A relatively simple procedure for the detection of specific anti-treponemal antibodies is the Reiter Protein Complement Fixation (RPCF) test. This test uses as antigen, a protein extract obtained from the Reiter strain or treponemes grown on artificial media. Unfortunately this method has fallen into disfavour for it is relatively insensitive particularly in early primary and late syphilis. False reactions may also occur due to non-specific inter-reaction with a lipopolysaccharide impurity contained in the antigen material[4]. These Reagin and RPCF tests remain popular because they are easily performed and quantitated and are inexpensive. They remain the standard for screening purposes and for following serological response to treatment. A titre of Reagin antibodies may be helpful in diagnosis but a rise or fall in antibody level over a period of time is more informative.

Distinction of whether the patient has a true treponemal infection or a false positive test is more reliably made by employing a specific treponemal test such as the Fluorescent Treponemal Antibody Absorption (FTA-ABS) or Treponema Immobilisation (TPI) tests. As both of these methods require a high degree of technical skill and rather expensive microscopic equipment with careful standardisation, they are not used in this country as routine diagnostic procedures for syphilis.

Because of these and other problems there is an obvious need for a simple and highly sensitive laboratory procedure to detect specific anti-treponemal antibodies. The *T. pallidum* haemagglutination assay (TPHA) described by Rathlev (1967)[9] and improved by Logan *et al.* (1970)[8] appears to have these features for it compares well with the FTA-ABS and is more sensitive than the TPI in primary syphilis.

The purpose of this paper is to compare the sensitivity of a simple micro-modification of the TPHA procedure[8] with the ETA-ABS test; to evaluate its potential for better interpretation of problematical sera and determine the stability of the reagents over a prolonged period of time.

Methods and Materials

Serum samples from a total of 220 patients were examined for the presence of anti-treponemal antibodies by the Micro TPHA procedure. Of these, 160 were initially tested by VDRL, quantitative Wasserman reaction[12], RPCF and FTA-ABS[13] methods, and shown to be reactive by one or more of these procedures. Many of these sera had been stored at minus 20°C before being examined by the Micro TPHA method.

A further 60 non-reactive sera were selected from patients with various diseases and tested by the Micro TPHA. A total of 30 sera were from glandular fever patients with strongly positive heterophile antibodies, 5 were from brucellosis cases, 10 from leptospirosis, 10 from various autoimmune diseases, 2 from hydatids and 3 from patients with hepatitis.

Micro TPHA

Reagents reconstituted according to the manufacturer's instructions (Fuzizoki Pharmaceutical) are as follows:

(a) *Absorbing diluent*: containing gum arabic, normal rabbit serum, Tween 80, sheep and ox cell membrane components, components of normal rabbit testes and cell components of Reiter treponemes all diluted in phosphate buffered saline pH 7.2.

(b) *Sensitised sheep cells*: formalised tannic acid treated sheep red cells sensitised with cell components of Nicholl's strain of *T. pallidum*.

(c) *Control sheep cells*: similar to (b) without sensitisation of *T. pallidum* components.

(d) *Reactive control serum*: a serum having TPHA level of 1:5,120.

All reagents when reconstituted were stored at 4°C unless being used.

Methodology

TPHA results were obtained using the 'Cooke Microtiter' system as directed by the Manufacturers' Instruction Manual (Cooke Engineering Co., Alexandra). Inactivated serum was initially diluted 1:10 in the absorbing buffer and left undisturbed for 30 minutes at room temperature. The diluted serum was then titrated in 0.025 ml volumes in 'U' shaped lucite plates from 1:20 through to 1:320 in the absorbing buffer. With each test serum a negative cell control was added to ensure removal of unwanted heteroagglutinins. The sensitised sheep cells were diluted 1:10 in absorbing buffer just prior to use and 0.025 ml added to each dilution of the test serum. The unsensitised sheep cells were treated similarly and 0.025 ml added to the cell control well. The lucite plates and contents were then mixed thoroughly utilising a 'Taiyo Bussan' micro mixer for 60 seconds. A preliminary reading was taken after 3-4 hours incubation at room temperature; a final reading was made after 18 hours incubation. A reactive serum showed a positive reaction of 1+ or more at a dilution of 1:80 and the titre of the serum was expressed as the reciprocal of the highest dilution showing a 1+ or greater reaction.

Results

A total of 30 serum samples containing high titre heterophile antibodies (1:160 or more) demonstrated micro TPHA levels of less than 1:40, showing minimal residual antibody to sheep red blood cells after 30 minutes absorption with the absorbing diluent.

The 30 serum specimens obtained from patients with various other diseases also showed little or no cross-reactivity with the micro TPHA procedure; the highest level (1:40) was obtained from a patient having leptospiral agglutinations of 1:6,400.

Figure 1 shows the typical haemagglutination patterns obtained using the 'Microtiter' system.

Analysis of serological results of 160 positive reactors is presented in Table I. A total of 55 sera having false reactive Reagin tests presented with non-reactive FTA-ABS and micro TPHA levels.

Eight sera considered to have falsely positive Reagin tests were reactive by the RPCF but non-reactive by the FTA-ABS and micro TPHA methods. Six sera giving negative Reagin tests but reactive by the RPCF were all non-reactive by the FTA-ABS and micro TPHA procedures.

Two patients considered on clinical grounds not to have treponemal infection gave repeated positive results by the FTA-ABS method but were consistently non-reactive by the micro TPHA procedure.

As demonstrated by previous authors[2, 8, 9, 11] correlation between the FTA-ABS and TPHA is excellent. In this series correlation of 92.5 percent was found.

One serum sample from a patient (E.D.) shown clinically to have syphilitic infection was

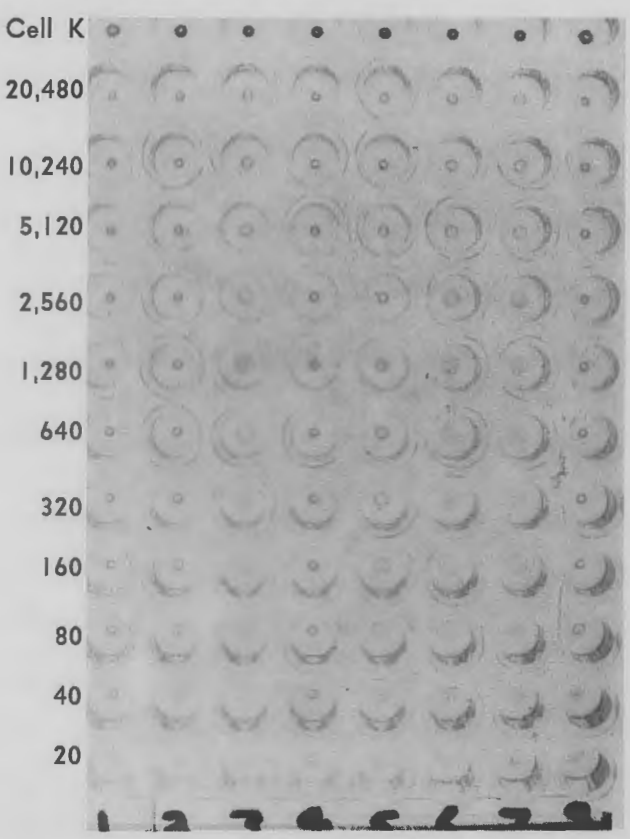

FIGURE 1.—Haemagglutination Results of Eight Sera with Varying Micro TPHA Antibody Levels.

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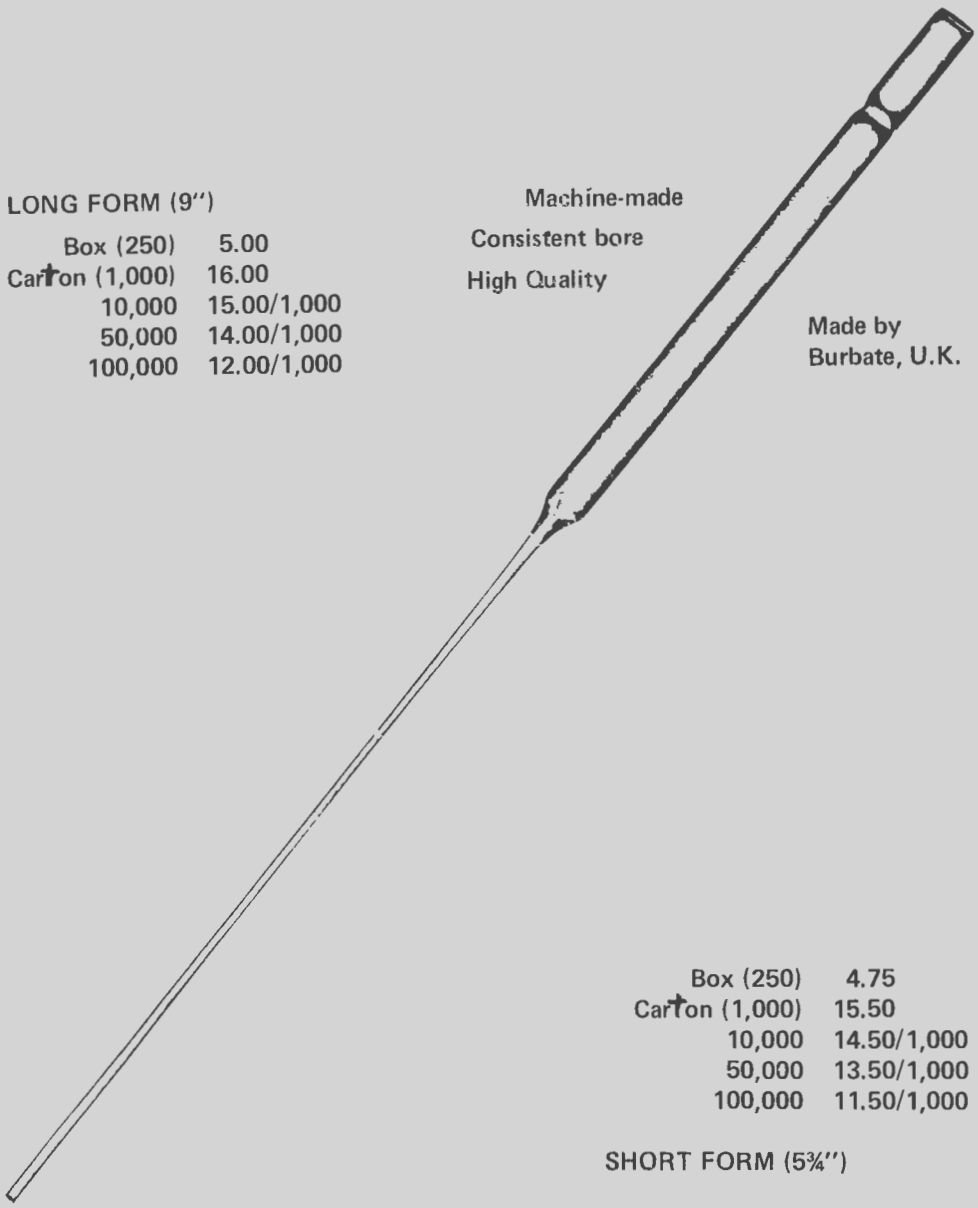

Box (250)	4.75
Carton (1,000)	15.50
10,000	14.50/1,000
50,000	13.50/1,000
100,000	11.50/1,000

SHORT FORM (5¾")

weakly reactive by micro TPHA (1:80) but non-reactive by the FTA-ABS procedure.

Seven treponemal infections were not detected by micro TPHA but were reactive by the FTA-ABS method.

Table I

Comparison of 160 Cases with Reactive Serological Results

False Positives

VDRL	WR	RPCF	FTA	TPHA	No. of Cases
±	±	N	N	N	55
±	±	+	N	N	8
N	N	+	N	N	6
+	N	N	+	N	1
+	A/C	A/C	+	N	1
21	47	16	2	0	71

True Treponemal Infection

VDRL	WR	RPCF	FTA	TPHA	No. of Cases
+	N	N	+	+	10
N	+	N	+	+	1
N	N	+	+	+	3
N	N	N	+	+	16
+	+	N	+	+	8
+	N	+	+	+	5
N	+	+	+	+	7
+	+	+	+	+	31
+	N	N	N	+	1
±	±	+	+	N	2
±	±	N	+	N	5
66	54	49	88	81	89

\+ Reactive.
± Either reactive or non-reactive.
A/C Anti-complementary.
N Non-reactive.

A significant proportion (36.4 percent) of treponemal infections in this series presented with reactive FTA-ABS and TPHA levels only.

Reproducibility studies on 10 replicate samples with varying TPHA titre levels showed an acceptable error factor of ±1 doubling dilutions. Twenty serum samples having reactive micro TPHA levels we re-examined using reagents that had been reconstituted for three months, these also demonstrated an error factor within acceptable limits, i.e., ±1 doubling dilutions.

Discussion

The micro TPHA method appears to be a simple, specific and highly reproducible procedure for detecting treponemal antibodies, having an excellent correlation with the FTA-ABS method. Because of its high degree of specificity it could well be utilised to discriminate biological false positive reactions from true treponemal infections, particularly where a false RPCF test has been demonstrated.

Reagents after reconstitution appeared quite stable in our hands for at least three months with little or no loss in reactivity. Because of this and a 10-fold saving in reagent consumption the micro TPHA procedure could be considered a desirable supplementary method for the serological diagnosis of syphilis.

Although the TPHA is not as sensitive as the FTA-ABS[2, 8, 9] method in primary syphilis it is capable of detecting some at a very early stage, for case number 25 (Table II) presented for laboratory investigation 14 days after contact.

The TPHA at this stage will not replace the FTA-ABS which is still the most sensitive laboratory test available for detecting treponemal antibodies at all stages of the disease. However, it far surpasses the RPCF test which in this series detected 54 percent of treponemal infections compared with 92 percent by the micro TPHA and might also be considered a useful adjunct in confirming positive FTA-ABS results.

Antibiotic therapy appears to have little effect upon both FTA-ABS and TPHA results once they are reactive, for they remain so even after treatment. It is likely, therefore, that neither test will replace quantitative Reagin methods for monitoring adequate treatment therapy.

False positive results by the FTA-ABS have been shown to occur and in this series two such reactions were demonstrated. One, showing atypical 'beaded' fluoresence, was from an active case of systemic lupus erythematosis The other was from a pre-natal patient, considered clinically not to have either a present or previous treponemal infection, who demonstrated a strongly reactive FTA-ABS result during the first trimester of pregnancy (confirmed by a Reference Laboratory).

Six weeks later the FTA-ABS was weakly reactive, three months hence non-reactive, without antibiotic treatment. Such false results have been recorded by Buchanan *et al.* 1970[1],

Table II

Typical Serological Results from 26 Sera.

No.	Sex/ Age	VDRL	WR	RPCF	FTA	TPHA	Diagnosis
1.	M/63	+++	N	N	N	N	Ca. prostate
2.	M/15	N	N	++++	N	N	Hepatitis
3.	F/36	N	64	N	N	N	Pregnancy
4.	F/26	++++	16	++	N	N	Discoid Lupus
5.	F/15	N	128	N	N	N	Atopic
6.	F/60	++++	32	+	N	N	Acquired Haemolytic Anaemia
7.	F/25	++++	16	+	N	N	? Primary Biliary Cirrhosis
8.	F/18	++	32	N	N	N	Pregnancy
9.	F/17	N	64	N	N	N	Non-specific urethritis
10.	M/55	+++	32	+	N	N	Cryofibrinogenaemia
11.	F/16	+++	A/C	A/C	+++	N	Systemic lupus
12.	M/35	++++	16	+	++	160	Yaws
13.	F/27	++	8	N	+++	640	Yaws
14.	F/25	N	N	N	+	160	Cook Islander
15.	F/25	N	32	+	++	320	Probable Yaws
16.	F/29	+++	8	++	+++	640	Yaws
17.	M/29	+++	16	+	++++	10,240	1° Syphilis
18.	M/18	++++	256	+++	++++	10,240	1° Syphilis
19.	F/21	N	N	+++	+++	5,120	2° Syphilis
20.	F/54	+++	32	N	+	Nil	1° Syphilis
21.	M/27	+	N	++	++++	1,280	2° Syphilis
22.	F/13	N	16	N	+	Nil	Early 1° Syphilis
23.	F/NB	++++	256	++++	+++	640	Congenital Syphilis
24.	M/20	N	N	N	+	320	Treated 1° Syphilis
25.	M/22	+	N	N	++	80	Very early 1° Syphilis
26.	F/44	N	N	N	+	160	2° Syphilis

N Non-reactive.
\+ Reactive.
++ Strongly Reactive.
A/C Anti-complementary.

Fischman, A. and Brec, A. S. (1971)[2], and Kraus *et al.* (1971)[7]. Both these cases demonstrated consistently non-reactive micro TPHA results.

Pathogenic treponemes of syphilis, yaws, pinta, bezel, or endemic syphilis are considered antigenically similar if not identical. At present there are no serological tests capable of differentiating the specific antibodies produced by these various diseases[6].

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The Erythrocyte Membrane, Its Cell Coat and Blood Group Antigen Anomalies in Cord Blood

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The functional importance of cellular membranes has led, especially during the last thirty years, to a lot of scientific investigation and subsequent data and discussion. The foresight of Davson and Dannielli, who in 1943 envisaged the cell membrane lipid as consisting of a bimolecular leaflet, brought new ideas and concepts to mind which in turn led to further investigation and subsequent illustration of the physiological function, and biochemical and histological structure of the cell membrane.

It is now generally agreed that the cellular unit membrane of most if not all cells is a bimolecular leaflet consisting of interlinking long chained fatty acids backed on two sides by a layer of protein. From electron microscopy studies evidence for this has been obtained by several workers[22, 21, 4]. The latest type of method employed is that of 'freeze etching' where cells are frozen by immersion in liquid nitrogen. Gentle tapping of the cell results in the cell membrane splitting along the line of hydrogen bonding within the membrane. The exposed faces are replicated by platinum-carbon shadowing while under high vacuum. By raising the temperature to —20°C the water of the membrane sublimes and the biological material removed by for example, household bleach. The carbon replica of the membrane face chosen is left and this is studied under the electron microscope. By means of this method artifacts produced by the fixation, dehydration, embedding and heavy metal staining of thin sections are removed.

The cell most commonly used for these studies is the human erythrocyte which has been shown to have a membrance variable in thickness at various regions of the cell[11], and varying in thickness from 50 Å to 1,000 Å dependent upon the preparation being of the wet or dry type. The erythrocyte has been shown to have a surface coat of mucopolysaccharide material enveloping the cell membrane.

Evidence has been obtained[15] to show that the 'cell coat' of cells is a secretion synthesised in the Golgi apparatus of the cell. This was done using the radio-activity labelled substrate galactose and following its progress within the cell using the electron microscope. The galactose was seen to be taken up to form glycoprotein within the Golgi apparatus and incorporated with the mucopolysaccharide complex of the cell coat. The mucopolysaccharide cell coat of the erythrocyte must therefore be present intact at an earlier stage of the cell's life, for example at the erythroblast stage of development, as the requirement for its synthesis is a functional Golgi apparatus and this is absent in the mature erythrocyte. Presence of the coat in erythrocyte precursors can be demonstrated by antigen-antibody studies[5]. As the coat is not renewed in the mature erythrocyte antigenic strength of the cell decreases with age.

The antigenic characteristics of the cell coat have been demonstrated in many body cells. One method used has been the incubation of fluorescein labelled antibody with cells and studying the cells upon subsequent histological sectioning[19]. Particularly the blood group antigens or substances have been found to exist widely in human tissue especially in epithelial tissue, vascular endothelium and erythrocytes[7, 20], and these blood group substances consist of repeating mucopolysaccharide units, the sugar present in a few of these substances being as follows[14, 18].

Substance	*Repeating Sugar Unit*
A	O ∝ N acteyl D galactosaminoyl 1-3 D galactose
B	O ∝ N galactopyranosyl D galactose
H	∝ L fructopyranosyl

Cell coats have been seen early in embryonic development[1]. A clear distinction between the integrated cell coat and a loosely adherent film of secreted material can be made if morphological studies are preceded by prolonged washing to remove particulate intercellular products and by attempts to differentiate the cell coats

and extracellular substances by specific enzymatic digestions.

Cell coats appear to be constantly and actively synthesised and the rate of membrane-coat development is increased in the cells of younger animals particularly those of the foetus and newborn[13, 12].

The study of the distribution of specific antigen sites in the peripheral cell coat of the human erythrocyte has shown a variation in number of antigenic sites for the blood groups studied[6]. The results obtained were as follows:

Cell	*Group A Sites Per Cell*	
Group	*Adult Blood*	*Cord Blood*
A_1	810,000 to 1,170,000	250,000 to 370,000
A_1B	460,000 to 850,000	220,000
A_2	240,000 to 290,000	140,000
A_2B	120,000	?absent

These results were demonstrated using ferritin, conjugated to the appropriate immunoglobulin which was specific for the antigen site under study, incubating the ferritin-antibody complex with the erythrocytes and counting the antigen sites using the electron microscope. The striking feature of these results is the decrease in antigen sites of the blood group antigens A_1 and A_2 in cord blood specimens.

Using a similar method, studies have been conducted on the uptake of ferritin into circulating erythrocytes by pinocytosis[2]. It was shown that ferritin uptake into the mature erythrocyte was dependent upon the presence of an antibody which had its corresponding antigen on the erythrocyte surface. But the ferritin molecule was pinocytosed as a ferritin-antibody complex only in the erythrocytes from cord blood, the resultant vesicles formed within the erythrocytes being counted using the electron microscope. Controls of the method used showed that antibody alone was pinocytosed by the corresponding antigenic erythrocyte. As this corresponding antigen on the cell surface was necessary for ferritin-antibody uptake, and also for antibody uptake alone, it can be concluded that as the antibody is bound to the antigen, the pinocytosed vesicles contain an antibody-antigen complex, with or without conjugated ferritin.

There is evidence therefore for increased cell coat and membrane turnover, decreased numbers of antigenic sites of blood groups A_1 and A_2, and antibody-antigen complexes being pinocytosed by erythrocytes in specimens of cord blood. All this throws light upon one of the problems associated with blood group serology.

Using serological techniques, numerous blood group antigens are found on the erythrocyte surface. Most of these antigens have been detected early in foetal life, a few examples from the literature are:

Blood Group System	*Foetal Age at Antigen Detection*
MNSs[17]	9 weeks
P_1[17, 8]	12 weeks
A[17]	37 days
Rhesus[16]	6 weeks

The blood group typing of cord and young infant blood samples in a few blood group systems is hazardous in view of the results obtained. These have been well documented. Details are summarised.

The blood group antigen A, may be subdivided in the majority of adults into A_1 and A_2 by means of the antisera anti A_1 (or α_1) and anti A (or α). Cells give the following results:

Anti A_1	*Anti A*	*Cell Group*	*Gene Frequency Phenotypes*
+	+	A_1	0.348692
—	+	A_2	0.09819
+	+	A_1B	0.025562
—	+	A_2B	0.008520

where + denotes reactivity between antigen and antibody and detected usually in the form of agglutination and/or haemolysis. With cord blood erythrocytes. the group A antigenic strength is decreased to such an extent that most specimens do not react with anti-A_1 resulting in A blood group subtypes being determined mainly as A_2 regardless of 'true' type[23]. The group A antigenic strength increases from birth, and between three and six months of age an infant will subtype for group A correctly[17, 6]. Of interest is the fact that at the stage of true group A subtyping the time lapse from birth is sufficient for one complete turnover of infant's erythrocytes, and also this time lapse is sufficient for the removal of any maternal antibody which may be circulating in the infant's blood system.

The blood group antigen P is similar to the A antigen in that results to determine cord blood erythrocytes as being of type P_1 or P_2 are unreliable[17, 8]. Once again the P antigen

increases in antigenic strength with age, and true typing is obtained between three and six months after birth. Of interest is the comparison of group P_1 antigenic strength which is strong in young foetuses but decreases with foetal age.

The Lewis blood group system is unique in that all cord blood erythrocytes group as Lewis (a-b-), that is the Lewis antigen is undetectable in these cells using techniques which normally would detect the presence of the antigen[17, 9, 3]. True Lewis typing develops at approximately three months of age[3], but varies during an individual's lifespan. The Lewis blood groups are now generally considered to be plasma groups rather than erythrocyte blood groups, the plasma antigen being absorbed into the erythrocyte surface to give the cell its specific Lewis antigenicity.

The two main erythrocyte blood group systems therefore which give unreliable subtyping results using erythrocytes from young infant or cord blood specimens are A and P. It is more than coincidence that their corresponding antibodies are perhaps the most common naturally occurring antibodies in man—55 percent of people have anti A in their serum, and possibly up to 25 percent have anti P_1 although this is normally present as a relatively weak antibody. These two antibodies, anti A and anti P_1, are of the 19S type immunoglobulin and this class of immunoglobulin has been generally assumed not to cross the placenta. However, their presence in cord blood has been confirmed by the writer and various authors[17, 16]. In cases of haemolytic disease of the new-born due to anti A the erythrocytes are spherocytic in shape and this is thought to be due to insufficient cell membrane production to maintain proper shape of the cell, and insufficient antibody present to cause premature removal of the cell by the reticuloendothelial system.

Inability to blood type confidently the groups A and P_1 has been proposed in the past as being due to the underdevelopment of the number of antigenic sites in the erythrocyte cell coat. This suggests that as the subject ages his ability to produce more developed antigen sites increases, and that the initial state of affairs is due to an underproduction of mature antigen sites by the cell and that this is somehow related to the immature metabolism of the cell possibly as a consequence of the immature metabolism of the infant as a whole. There is, however, enough evidence available to suggest that the antigen sites of the blood groups A and P_1 are well developed from an early foetal age, and as a consequence of maternal antibody of type anti A and/or anti P_1 crossing the placenta in small quantities, insufficient to cause haemolytic disease of the newborn, the erythrocyte A and P_1 antigens are pinocytosed as an antibody-antigen complex by the erythrocyte thereby resulting in fewer antigen sites on the surface of the cell leading to the lack of reactivity with the appropriate antisera.

It would be interesting to confirm this theory of loss of antigenicity due to pinocytosis. This could be achieved by quantitating the amounts of antibody present in cord blood specimens and relating the results obtained to the antigenicity of the corresponding cord blood erythrocytes. Quantitation of antibody could possibly be obtained by means of a similar method to that of using the automatic technique of the Autoanalyser (Technicon) for the amount of Rhesus anti D antibody present in mothers sensitised to the D blood group antigen. One would expect to find a loss of antigenic activity of type P_1 or A in cases where the appropriate maternal antibody is present in the infant's serum. Comparative studies on cord blood erythrocyte antigenicity in cases of maternal-infant ABO or P blood group compatibility and incompatibility would demonstrate whether or not the loss of erythrocyte antigen sites in the infant was related to the corresponding antibody being present in maternal serum. Studies using the electron microscope to confirm erythrocyte vesicle formation relating to antigen-antibody pinocytotic activity would also be helpful as would comparative studies of the activity and sugar uptake of the Golgi apparatus in adult and newborn erythroblasts.

Although perhaps not of great practical significance, the inability to subtype young infants for blood groups A and P requires further investigation as the concept of immaturity of erythrocyte antigen sites in the newborn appears to be incorrect on the basis of present evidence.

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Species Identification of Klebsiella Isolated from Sputa

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Winning Entry, Trainee Essay Award (Technical Section) 1972

Introduction

The pathogenic role of members of the Klebsiella genus in pulmonary infections is uncertain. However, certain species of the genus, namely *K. edwardsii-edwardsii, K. edwardsii-atlantae, K. pneumoniae* have been suggested as primary pathogens[6]. *K. aerogenes* is a common isolate in sputum, and is generally accepted as being of doubtful pathogenicity and is not infrequently observed following antibiotic therapy. In this respect *K. aerogenes* may fulfil an opportunistic role. It would seem therefore that speciation of such isolates is of some importance as established by Foster and Bragg[9] as pathogenicity within the genus varies greatly. The significance of *K. rhinoscleromatis* and *K. ozaenae* isolates, probably because of their infrequent isolation, is difficult to assess.

In the present study 40 strains of Klebsiella isolated from sputa over a 13-month period (April 1971 to May 1972) were investigated biochemically.

Material and Methods

The organisms were considered possible Klebsiella if they were non-motile Gram negative bacilli which failed to 'decarboxylate' ornithine decarboxylase broth. All sputa received were homogenised using the pancreatin-trypsin method of Rawlins[12], and cultured on Blood, MacConkey and Chocolate Agar and incubated in a 5 percent CO_2 atmosphere for 18-24 hours at 37°C.

Motility: The improved method of Ball and Sellars, was used.

Urease: Christensen's medium without agar was used[10].

Citrate Utilisation: Kosers citrate broth and Simmons citrate agar were tested in duplicate and no discrepancies found.

Methyl Red (M-R) and Voges-Proskauer (V-P) Reactions: Isolates were seeded into 0.5 ml aliquots of glucose phosphate broth and incubated at 37°C for 24 hours. The micromethods of Barry *et al.*[2] and Benjaminson[3] were checked using the macro method[5] and exact correlation was obtained in each instance.

Lysine Decarboxylase: Lysine Iron Agar (Edwards and Fife[8]) was used instead of the decarboxylase broth.

Ornithine Decarboxylase: Moellers[11] decarboxylase broth was used.

Miscellaneous: Indole production, gluconate oxidation; glucose, lactose and dulcitol fermentation tests were carried out by approved methods.

Results

The results obtained are shown in Table 1.

Table 1

	K. aerogenes		*K. pneumoniae*		*K. ozaenae*		*K. edwardsii-edwardsii*		*K. edwardsii-atlantae*		Total	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Indole	9	19	0	4	0	6	1	0	1	0	11	29
Ornithine	0	28	0	4	0	6	0	1	0	1	0	40
Motility	0	28	0	4	0	6	0	1	0	1	0	40
Gluconate	25	3	1	3	0	6	0	1	0	1	26	14
M-R	0	28	4	0	6	0	1	0	1	0	30	10
V-P	28	0	0	4	0	6	1	0	1	0	12	28
Glucose (acid)	28	0	4	0	6	0	1	0	1	0	40	0
Glucose (gas)	28	0	4	0	5	1	0	1	1	0	38	2
Lactose (acid)	28	0	4	0	1	5	1	0	1	0	35	5
Dulcitol (acid)	16	12	4	0	0	6	0	1	0	1	20	20
Urea	28	0	4	0	3	3	1	0	1	0	37	3
Citrate	27	1	4	0	5	1	1	0	1	0	38	2
Lysine	28	0	4	0	1	5	1	0	1	0	35	5

Of the 40 isolates 70 percent were *K. aerogenes* which were differentiated from other isolates by their negative M-R and positive V-P reactions. The remainder consisted of *K. pneumoniae* (10 percent), *K. ozaenae* (15 percent), *K. edwardsii-edwardsii* (2.5 percent) and *K. edwardsii-atlantae* (2.5 percent). These were identified by their positive M-R reaction and differentiated by acid production in dulcitol, gas production in glucose, lysine decarboxylation and gluconate oxidation.

Discussion

Traditionally inclusion of enterobacteria into the Klebsiella genus has been based on negative indole production and M-R reaction, and positive V-P and citrate utilisation reactions. The results gained in the present study indicate that these criteria are insufficient as 30 percent of the Klebsiella identified were found to be M-R positive. Reference to Table 1 will show that the majority of Klebsiella isolates may be identified generically by testing for citrate utilisation (positive), urease activity (positive), ornithine decarboxylation (negative) and motility (negative). It is therefore suggested that these tests could usefully replace those within the IMVIC series.

The large occurrence (28 percent) of Klebsiella which are indole positive show that erroneous results may be recorded when only an indole is performed as a preliminary screening procedure on lactose positive, Gram negative bacillary isolates. To cover this anomaly a minimum battery of indole, ornithine, motility and urea tests should be performed on all lactose fermenters. Any organisms which do not conform should be subjected to further biochemical study.

Alhough gluconate is positive for the majority of *K. aerogenes*, it is negative for the other species. Urease production was found to be more reliable in that 92.5 percent of the total isolates were positive while gluconate was oxidised by only 65 percent.

Once the organism is classified as a Klebsiella, it has been found difficult to further differentiate within the genus. However, the biochemical classification of the Klebsiella as proposed by Cowan *et al.*[4] and confirmed by Foster and Bragg[9] has been confirmed in this study and is useful in identifying respiratory Klebsiellae. It would appear that differentiation within the Klebsiella genus as advocated by Darrell and Hurdle[6] is important and especially in respiratory disease where a report of Klebsiella species may be misleading.

Attempts were made to serologically confirm the biochemical groups using polyvalent 1-6 Klebsiella and types 1-6 specific antisera, by means of the Quellung reaction and slide agglutination reactions[7]. However, these have so far proved unsuccessful.

Summary

Forty strains of Klebsiella isolated from sputa have been assigned to this genus and specified by means of biochemical properties. It is suggested that all respiratory Klebsiella, have a minimum of indole, ornithine, motility, urea M-R and V-P tests, and further biochemical studies should be carried out on all M-R positive organisms.

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Lipoprotein Electrophoresis on Cellulose Acetate

R. W. L. Siebers, ANZIMLT, and K. Clarkson, FNZIMLT

Pathology Department, Hutt Hospital

Received for publication, August 1972

Introduction

Lipoprotein typing has been performed using different media for electrophoresis. It was developed by Lees *et al.* (1963)[1] using paper and was subsequently developed with other media such as agarose gel, polyacrylamide gel and cellulose acetate.

Agarose gel clearly separates the important beta and pre-beta bands while the chylomicrons remain as a sharp band at the origin. However, preparing the gel slides is tedious and commercial slides are expensive. Polyacrylamide gel requires prestaining with subsequent masking of of the chylocicrons. It does not possess the required porosity for the very large pre-beta molecules and so inverts the beta and pre-beta bands. Cellulose acetate exhibits excessive chylomicron migration and strongly absorbs lipid stains.

After experimenting with different types of cellulose acetate and lipid stains we have concluded that good results can be obtained by using gelatinised cellulose acetate and an improved Oil Red O stain.

Reagents and Instrumentation

1. Oil Red Om. obtained from Helena Laboratories, Beaumont, Texas.
2. Tris, barbital, sodium barbital buffer; pH 8.8, ionic strength 0.05. Dilute one sachet powder to 1,000 ml with distilled water. Prepare fresh every two weeks. This was obtained from the same source as the Oil Red.
3. 1 N NaOH.
4. Cellogel 200 cellulose acetate. (Chemetron, Milan, Italy.)
5. Di-ethylene glycol.
6. Beckman microzone cell, model R-101. (Beckman Co. Instrument Inc. Ltd., Fullerton, California, USA.)
7. Microzone densitometer, model R-110. (Beckman Co.)
8. Sample applicator. (Beckman Co.)
9. VoKam power supply. (Shandon Scientific Co. Ltd., London, GB.)

Method

Fasting blood specimens are obtained, allowed to clot and the serum separated. Electrophoresis is carried out within six hours.

The cellogel strip is washed in running tap water to remove excess methanol and soaked in the barbital buffer for 15 minutes. It is gently blotted between filter paper to remove excess buffer and placed in the microzone cell. Two applications of serum are applied with the applicator (0.50 μL) on the middle of the strip and electrophoresis is carried out for 15 minutes exactly at 250 V.Bridge gap 6.5 cm.

During electrophoresis 6 ml of Oil Red Om is mixed with 1 ml of 1 N NaOH and 0.5 ml di-ethylene glycol in a petri dish. On completion of electrophoresis the strip is left to soak for 60 minutes in this mixture, after which it is washed under running tap water and excess stain is removed from the strip by gently wiping with a wet piece of cotton wool.

The strip is then placed in a plastic folder, covered with a thin layer of water and scanned on the densitometer.

Discussion

Good separation of the different lipoprotein fractions is obtained as shown in Figure 1.

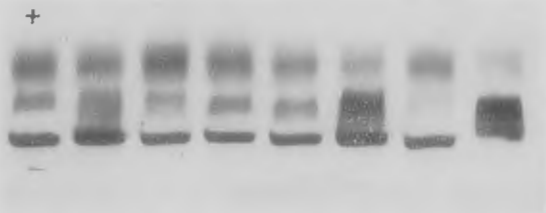

FIG. 1.—Typical example of a daily run.

In our laboratory we estimate the beta and pre-beta fractions from the densitometric scan and total lipid estimations. (Colorimetric sufo-phospho-vanillin.) Objections to densitometric determination of lipoproteins are based on the non-linear relationship between concentration and density of bound dye reported by Swahn (1953)[4]. However, a good correlation between densitometric scan and ultracentrifugal analysis has been observed by Noble *et al.* (1968)[2], (1969)[3].

Di-ethylene glycol has been included in the colour reagent as it gives a clearer background. Hesserschmidt *et al.* (1972)[5].

The same buffer and electrophoresis cell are being used by us for protein and LDH isoenzyme electrophoresis.

Summary

A simple and rapid method of lipoprotein electrophoresis utilising gelatinised cellulose acetate and Oil Red Om has been described, giving clear-cut separation and staining.

Acknowledgments

We wish to thank Dr J. F. McCafferty for his helpful criticism of the manuscript and Mr R. B. Sparke for the photography.

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Technical Communications

Toluidine Blue Stain for Acid Mucopolysaccharide Fibres in Sputum

Sir,—Bromhexine Hydrochloride ('Biosolvon'), a synthetic substance related to vasicine, an alkaloid derived from the plant *Adhatoda vasica,* is receiving increasing attention as an expectorant and antitussive. Bromhexine fragments acid mucopolysaccharide fibres in sputum and should therefore allow easier expectoration of sticky tenacious sputum. It should be noted that infection can also cause fragmentation through breakdown by bacterial enzymes[1].

The effect of Bromhexine on acid mucopolysaccharide fibres (A.M.P.S. fibres) can be demonstrated by staining smears of fresh sputum with Toluidine Blue and examining under polarised light[1, 2] when fibres are seen as long

thick continuous bundles or sheets of fibre, yellow to gold in colour against a dark background. After fragmentation, either by bacterial enzymes or Bromhexine, the fibres appear as diffuse yellow points. During intermediate stages of fragmentation, the thick bundles of fibre become thinner and break down into smaller but still discreet fragments.

Recently a double blind clinical trial of Bromhexine was carried out at Green Lane Hospital by Dr K. J. Thompson[3] during which sputum smears from each patient in the trial were regularly stained and examined. At the beginning of the trial, all smears were stained by the method described by Bürgi[2]. Smears were prepared from fresh sputum specimens by squashing a portion of the specimen between two glass slides which were then drawn apart. The smears were air dried, fixed in equal parts of ether and alcohol for thirty minutes and stained for three minutes in 1 percent aqueous Toluidine Blue pH 6.0. This was followed by two or three rinses in alanine-alcohol (10 mls alanine in 90 mls, 96 percent alcohol.) After which the smears were washed once with each of the following: Eucalyptus Oil, Carbol-Xylene (10 percent phenol in Xylene) and Xylene. The stained smears were dried and mounted in Gurr's 'Depex' and examined under polarised light.

Smears stained as described above were very variable in quality. In some the A.M.P.S. fibres were easily seen and in others they were very difficult to distinguish, being clearly seen only in occasional small areas, making estimation of the presence and extent of fibre fragmentation almost impossible. These smears appeared to be insufficiently decolourised and the fibres obscured by excess Toluidine Blue. After some experimentation, it was found that if the smears were washed thoroughly with tap water, both before and after the three analine-alcohol rinses, then the problem appeared to be overcome. The colour of the smear after the second tap water rinse and after staining is completed, should be purple rather than blue.

When the variation in quality of the staining of the first set of smears became apparent, duplicate sets of smears from each specimen received were made, only one of which was stained. When the staining method had been modified satisfactorily, the second set of smears was stained and compared to the first. In every case the fibres stained by the modified method were very much more easily seen, especially when in early or complete stages of fragmentation.

Jane Reeve,
Microbiology Department,
Pathology Department,
Green Lane Hospital,
Auckland.
June 9, 1972.

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Identification of *Allescheria boydii* from a Case of Maduramycosis

N. Dennis

Southland Hospital, Invercargill

From a paper read at the South Island Seminar of the NZIMLT, Timaru, April 1972

Maduramycosis is a chronic infection of feet and rarely hands and other parts of the body caused by a variety of filamentous fungi belonging to different species of Genera and characterised by the development of tumefactions and sinuses. The disease progresses slowly and the mode of onset is neither characteristic nor uniform. It can start as a small papule which later develops into an abscess which can rupture and form as a fistula. As the infection extends deeper into the tissues, the muscles, bones, fascia, and tendons may become involved and foot or hand becomes club-shaped or may develop into a mass of two or three times the normal size. Nodules frequently develop about the openings of the fistula from which an oily fluid drains with the diagnostic granules.

Numerous fungi that have been isolated from cases of Maduramyocosis fall into two

classes—ascomycetes and fungi imperfecti. In the ascomycetes are found the Genera Allescheria, Aspergillus, and Penicillium, and in the fungi imperfecti are found species of the genera Madurella, Indiella, Cephalosporium, Glenospora, Phialophora and Monosporium.

In February 1972 *Allescheria boydii* was isolated from a case of Maduramycosis in the Bacteriology Department of Kew Hospital. It was isolated from the foot of a 13-year-old female from the Gilbert and Ellice Islands. The specimen we received was an oily exudate aspirated from a deep-seated fistula. The fluid was blood stained with a few fibrofatty tissues. With careful examination yellow-coloured grains were picked up from it and examined under the microscope after placing them in a drop of 10 percent KOH. The granules were oval, irregularly shaped mass of 0.5 to 2 mm—composed of a central mass of segmented branched hyphae with many large chlamydospores. Around the periphery the hyphae assumed a radiating appearance. Plenty of granules were seen throughout the mass.

A few of the granules were aseptically washed in sterile normal saline and cultured on Sabourauds dextrose agar and mycosel agar. They were incubated at 28°C.

A rapidly growing fungus producing a white cottony aerial mycelium was noted on these two plates within a week. The culture on mycosel started showing better growth which later produced grey pigments. Microscopic examination after 21 days showed ovoid and pyriform asexual conidia eight to ten microns long by five to seven microns wide. They were produced singly at the ends of long conidiophores and on the sides of mycelium on short conidiophores. At this stage the culture was considered as an imperfecti form and termed *Monosporium apiospermum*. The plates were reincubated and a subculture was forwarded to Mr Rush-Munro for confirmation. The plates were periodically examined and at the beginning of the sixth week after incubation dark-brown thin walled Perithecia or Cleistothecia 50 to 200 microns in diameter were produced in the culture. These were visible macroscopically. On microscopic examination it showed asci and ascospores emerging from the ruptured perithecia. The elliptical ascospores were 4 to 4.5 microns × 5.5 to 7 microns in size. The production of Perithecia established the identification of the fungus as an Ascomycetes—*Allescheria boydii* which was confirmed by Mr Rush-Munro. He also noted in his report that this particular species showed thiamine as a nutritional requirement. This statement tallied with our finding of the rich growth of *Allescheria boydii* on mycosel agar compared with sabourauds dextrose agar. Mycosel agar contains phyton peptone—a rich vitamin complex containing thiamine.

Maduramycosis rarely heals spontaneously and the disease continues to progress and the patient eventually dies of secondary infection. To prevent this, below the knee amputation was conducted on this patient which was followed by antibiotic treatment for further prevention of secondary infection.

TECHNICAL COMMUNICATION

Scanning Accessory for Unican SP800 Recording Spectrophotometer

Sir,

Faced with delay in obtaining equipment for scanning and integration of serum electrophoretic strips the scanner described below was developed and has been used successfully for two years at this laboratory.

The chassis for the unit was made from 16 gauge sheet aluminium and incorporates a 5 cm wide section which fits the cuvette holder slide at the right of the cell compartment. A stand mounted on this chassis supports the constant temperature cell housing thus avoiding the inconvenience of disconnecting the water circulation tubes. Cellulose acetate strips are supported on a carrier by means of a 75 × 50 mm microscope slide held by spring steel clips. Two aspects of manufacture require precision. It is essential that the 3.5 mm wide slot in the carrier has parallel edges to ensure a uniform hori-

zontal baseline in scanning. Also the position of the strip being scanned should be at the narrowest point of the light beam. In our instrument this is 14 mm from the right-hand wall of the cell compartment. This can readily be located with a transluscent screen such as tissue paper.

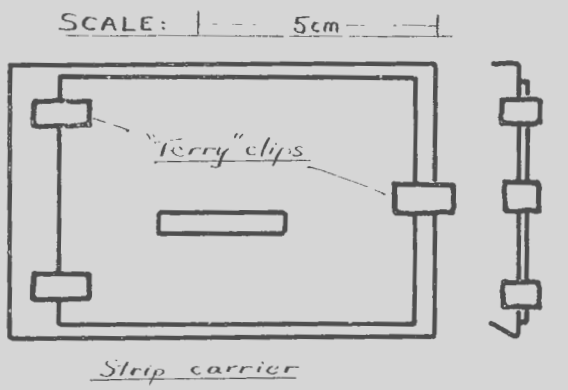
Linear drive for this carrier was provided by a simple clockwork laboratory time. An extension of the handpiece was used to move the strip carrier. It is accepted that on account of this drive system sweeping an arc, the speed of scan will vary slightly according to the position of the radius arm. As the length of arc employed relative to the radius of the drive arm is small this variation is considered to be insignificant.

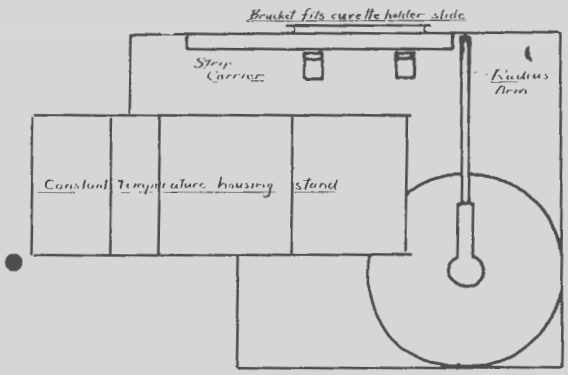
The clockwork drive is automatically wound in moving the drive arm prior to placing the strip carrier in the unit. A conventional scan is obtained. In the absence of an integrator the technique of weighing paper sections cut from these scans has proved to be a convenient means of quantitating protein fractions. Like other integrating systems, results obtained are directly related to the quality of the original electrophoretogram.

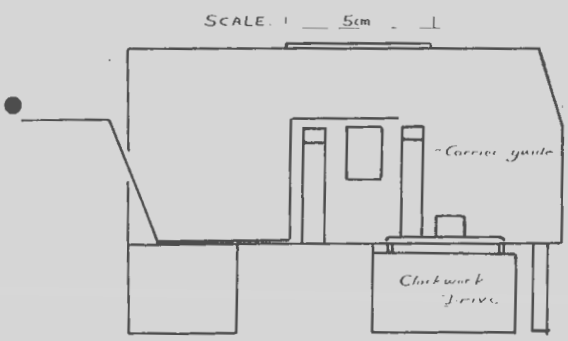
This accessory has had ready staff acceptance and has served a useful purpose prior to obtaining the scanning and integration equipment which is currently being commissioned in this department. A more sophisticated version would appear to be a worthwhile addition to the accessories offered by this company.

D. A. McArthur,
Pathology Department,
Middlemore Hospital,
Auckland.

August 25, 1972.







CORRESPONDENCE

Sir,

I was surprised to learn at the chemical pathology forum at Conference this year, that many people experienced difficulty in getting sulphanilic acid to dissolve when preparing the diazo reagents for bilirubin estimation. We find that solution is easily effected with the aid of a magnetic stirrer.

MRS J. PARKER,
Diagnostic Laboratories,
Dunedin Hospital.

September 28, 1972.

Sir,

Judging by the sedated atmosphere of the Annual General Meeting at Conference, Bernie's 'Young Turks'[1] must have been smoking pot in their hookahs.

The far-reaching and important issues of education and registration with all their ramifications which are going to radically affect the prospects and future of the young technologist were passed over in glassy silence. There was surely scope here for spirited debate. There is certainly need to consider the method of electing Councillors, which on a regional basis is restrictive and can prevent worthy members standing for office. This point was well made and is to receive Council's attention, but I would feel less apprehensive about the future of medical technology if there were some signs of intelligent interest in, and some critical appraisal of the business being discussed.

—'Old Turk'.

1. *Mr Collins speaking to a motion relating to the reform of Council elections, referred to the need for some 'Young Turks'. This expression has come to signify the replacement of the old brigade, regime or establishment by a younger, fresh blooded 'with it' group bursting with contemporary ideas. The expression is quite old and originally referred to the 'Young Turks' party which in 1909 attempted to check national decline by a revolt which instituted constitutional government.*—Editor.

Obituary

Harry Edwin Foster

As I remember, I met Harry Foster on HM Transport 'Empress of Britain', which in 1940 was taking the staff of 1NZ General Hospital supposedly to the Middle East as part of the Second Echelon. Harry was the laboratory staff for 2NZGH but was 'attached for training'. His laboratory experience at that time had been mainly with animal care, at Manchester 1934-38 and at Wallaceville 1938-40. The Second Echelon was diverted to England and I have a photograph of Harry on the steps of the laboratory in the tiny guard house at the gates of Pinewood Hospital, Hampshire. He continued to serve, later with the rank of Corporal, in the laboratory 1NZGH at Heliopolis, and later Helwan, in Egypt, and during its all too brief sojourn at Pharsalos in Greece. During the hurried evacuation Harry carried one of the microscopes and his heart was almost broken when the navy refused to allow it aboard the rescuing craft. He later worked with 2NZGH and on return to New Zealand in 1945 joined the staff of the Pathology Department, Christchurch Hospital, where he completed his training and gained the COP in 1950. The same year he joined the private Pearson Laboratory. There he worked eight years before going, after brief periods at Ashburton and in Dr W. S. Alexander's Lower Hutt Laboratory, to Taumarunui at the end of 1961.

His educational background and early training were limited, but by hard work he became fully qualified and a Charge Technologist, running a good country town laboratory. It is hard to imagine a more faithful and willing worker.

He is survived by his wife, and a son and a daughter by a previous marriage.

—D. T. S.

"Lab-line"

Have you an insoluble problem? Something on your mind? Do you wish to advise, admonish, reprove, reprimand, complain or congratulate? This column is for controversy!

Vacuutainers and Risk of Hepatitis

Department of Health Circular No. 1971/90 states, 'In view of the fact that the main risk of infection is from aerosols and that vacuutainers are considered likely to produce aerosols, the committee recommended that the practicability should be investigated of using disposable syringes for such blood collection with such disposable containers as are suitable and available.'

Contradicting this, the memorandum from the advisory committee on laboratory services in the Auckland Hospital Board states, 'The caps of vacuutainers often become contaminated with blood during collection. *However, the advantages of vacuutainers appear to outweigh the disadvantages provided multipuncture needles are used when necessary.* (Their emphasis.)

We use vacuum collection tubes, without multipuncture needles which are very expensive. Hundreds of thousands of these tubes are used yearly. We all have our views on which is the better system but it would be helpful if the Health Department would initiate some research in this subject and call for a report in say, four weeks. If we are to have advice, then let it be clear and unambiguous. Also, I do not wish to contribute to the barely concealed hysteria on this subject, *but cigarette smoking in laboratories should clearly be forbidden!*

Multiple Cell-counting Chambers for Urines

This subject arose at the 'Beecham's Seminar' in Auckland (organised, it seems, for the simple purpose of telling us politely and correctly, that many of us do not know how to read penicillin and other sensitivities). *Professor Smith was quite wrong when he said that doing say, two hundred urines in two place counting chambers isn't tedious. He obviously doesn't do them.* I have tried to interest ERMA of Japan in producing a multiplace counting chamber but have had some communications problems. Meanwhile I note that an article has been published in Medical Laboratory Technology describing such a tool, although it was unruled. I have referred this article to Beechams in the hope that they will shortly announce the production of a 10-place, simply ruled plastic disposable counting chamber!

Spectrophotometer Lamps

There is a remarkable similarity between the tungsten lamp costing over $7 and Lucas power bulb marked S.C.C. 12V 36W No. 2 at 32 cents. It is a very simple matter to transfer the collar and lead from an old lamp to a Lucas replacement. A simple wooden jig for mounting the collar can be constructed in five minutes and 12 bulbs can be changed over in an hour. We have never had an accident, but safety goggles and a cloth over the bulb are advised when removing the collar.

A. MILNE,
Whakatane Hospital.
June 9, 1972.

'The commencement of a Journal is never a step to be undertaken lightly—however, it was the unanimous opinion of those present at the first Annual General Meeting of the Association, held in Wellington, that a Journal was a necessity as a means of keeping all the members of the Association acquainted with the progress of their fellow members and the dissemination of all knowledge thought to be of interest and use. The progress of the Journal and its value will, however, depend on the active support of all members, for material to publish . . .' This quotation is from the first Journal editorial, April 1946. At the third annual conference in 1947 the Editor presented the Journal Report and lodged an earnest appeal for material. Finally a quotation from the editorial of October 1949, Vol. 4. No. 4.

'At each Conference the Editor is heartened by the flattering references to his ability and overwhelmed by offers of assistance; unfortunately, these are mere words and are rarely followed by articles.'

Things have not improved. I wrote to more than 20 people presenting papers at Conference pointing out that there is a long-standing request that such papers be presented for possible publication. *Two people favoured me with the courtesy of a reply.*

—R. D. ALLAN.

Book Reviews

Elementary Calculations in Clinical Chemistry. G. R. A. Padmore. 126 pages. Tables of logarithms and square roots. 1972. Published by Churchill-Livingstone. Price, £UK1.25.

This little book is designed specifically for students pursuing medical laboratory science and biology courses whose studies are handicapped by a lack of confidence in their ability to perform calculations. This applies to most of us.

SI units are extensively, but not exclusively, used throughout and if the questions which terminate each chapter are worked through, the necessary facility in dealing with these units should be acquired.

The chapters cover SI units, laws of behaviour of gases and solutions, volumetric analysis (at some length), colorimetry, hydrogen ions and buffers and some aspects of statistics.

The justification for the use of moles rather than milli-equivalents or normality through the use of a formula which considers the stoichiometry of the reaction is given. (The concept of equivalent weights and the use of mill-equivalents still has, to my mind, a pleasing simplicity and certainly is useful for acid base correction therapy.) The volumetric chapter includes a precise exposition of the mechanism involved when EDTA is used for chelating titrations and this includes the structural formula. In general there are many practical examples relating to laboratory tests.

Spectrophotometry includes a brief explantion of the use of molar and specific coefficients with rather simple examples. The problems are somewhat less difficult than those posed for the benefit of our final examination candidates. The other chapters also adhere to essentially simple explanations of the necessary theory. pH is mathematically defined and the interconversion of pH and hydrogen ions exemplified, buffers are explained in relation to the dissociation constants of weak acids, but the concept of conjugate acids and bases does not receive specific mention.

Statistics covers all the simple concepts inluding the 't' test but not correlation coefficients, for example. There is no easy way to absorb information even if collected in a convenient form as in this book. It has to be painstakingly and methodically acquired. Somewhat paradoxically our examination candidates would have to learn to cope with examples of rather more complexity than those exemplified. With this proviso I recommend this book to those who lack confidence in their calculating ability.

R.D.A.

Textbook for Laboratory Assistants. First edition. Irwin A. Oppenheim. C. V. Mosby Company (1972). 149 pages of text, 79 non-coloured illustrations. Price, $NZ4.90.

The book as the title suggests is for laboratory assistants and it indeed makes no pretention to go beyond the required level. But it does give the impression of stimulating ideas for furtherance of knowledge.

The contents are in eight chapters. Chapter One, of 30 pages, deals almost too briefly with fundamentals. Basic physiology and anatomy, simple mathematics, quality control, instrumentation and laboratory skills are all attempted to be covered. I personally feel that insufficent coverage is given to these essentials which should be fully familiar to the student. Indeed, even at the expense of later chapters, a greater depth should be given here. But the section has a redeeming feature, the first-class, simple line illustrations.

Chapter Two (22 pages): urinanalysis and other routine analyses fare well on examination. Urine chemical constituents and crystal structures are dealt with adequately and certainly written in a level of language comprehensible to the average student.

The other six chapters, clinical microbiology, serology, haematology, blood banking, clinical chemistry and clinical procedure are approximately equally divided in coverage and quality of comparison. A laboratory assistant who specialised in one discipline would easily be able to grasp from the text a simple working knowledge of another. Certainly working methods are not stressed but the all-important principles are clearly stated.

This textbook, although similar to several others in its broad spectrum approach, does

succeed in implanting a basic theoretical and working knowledge of the laboratory as a whole. Indeed it supplies the eye for the hook to be attached to during that first year of service.

G.H.T.

Malignant Blood Diseases—First edition 1972, by R. L. Sewell, FIMLT. Published by Bailliere Tindall, London. Price £UK2.50.

This book of 133 pages is one of a series of laboratory monographs. In its preface it is described as 'an attempt to provide senior technicians, pathologists and research workers with a concise account of the laboratory investigation of the malignant blood disorders.'

It is divided into four chapters. The first deals with the classification and diagnosis, and describes the haematological findings, including some cytochemistry, of the major disorders.

Chapter two deals with disease metabolism, including RNA, DNA, protein synthesis, cell life span, abnormal haemoglobins, and a small section on coagulation results which may be found.

Chapter three delves into the hotbed of immunology, including a detailed description of various methods, and some typical findings.

Chapter four covers cytochemistry methods and findings; these include some not usually found, such as leucocyte zinc, but excludes peroxidase and Feulgen stains. There is a small section on chromosomes and a list of their significance in some of the diseases mentioned.

The book is set out well, but one feels it is too full of technical detail and methodology for its size and object. It certainly covers a great deal of ground, some of it obscure. One also feels that some of the statements expressed are really the author's opinion rather than established facts. As an example it states 'Myeloma is less common than the finding of a benign monoclonal gammopathy characterised by an abnormal band in the serum of normal individuals'. It is now well established than 70-80 percent of all abnormal bands are in fact associated with some malignant process.

Illustrations are in black and white, and suffer in detail as a result. There is really little to be gained from their inclusion.

In summary this is a book which I feel would be useful and of more help to students, those preparing for examinations, or establishing techniques than those for whom the preface states it was intended.

It would be worthwhile as a quick and easy reference on results likely to be obtained in malignant diseases.

—C. S. S.

Tissue and Cell Culture. A laboratory monograph. Andrew Whitaker, BSc. PhD. 119 pages. Bailliere Tindall, London WC2 8QE. Price £UK2.50.

The author declares that the aim of this monograph is to describe current theories and methods of tissue and cell culture. It was his object to demonstrate that, not withstanding its contribution to other disciplines, study and practice of cell culture is a science in its own right, and has a most valuable part to play in biology as a whole. I think that it is undoubtedly fair to say that the aims and objects of the author have been achieved. There have been many books written on this subject in the last decade and much of the information given can be found elsewhere but rarely can one find collected together in one place the considerable information contained in this book.

The text is arranged in a very logical order and would lead anyone with the least interest in the science to an enthusiasm for futher reading.

It is evident that the author's primary interest is the use of tissue and cell culture in virology and the emphasis is somewhat in this direction. The indication, however, of the association of tissue and cell culture with other fields is sufficient to stimulate the enthusiasm of any newly qualified technologist with an inquiring turn of mind.

The early sections of the book dealing with the basic considerations of tissue and cell culture while outlining some of the problems which are met, gives not only the reasons for the problems but the means of avoiding them.

I would recommend the book to any senior technology trainee who requires compact and up-to-date information on this subject, and in particular I would recommend it to any whose scientific interest is tissue and cell culture.

—H. E. H.

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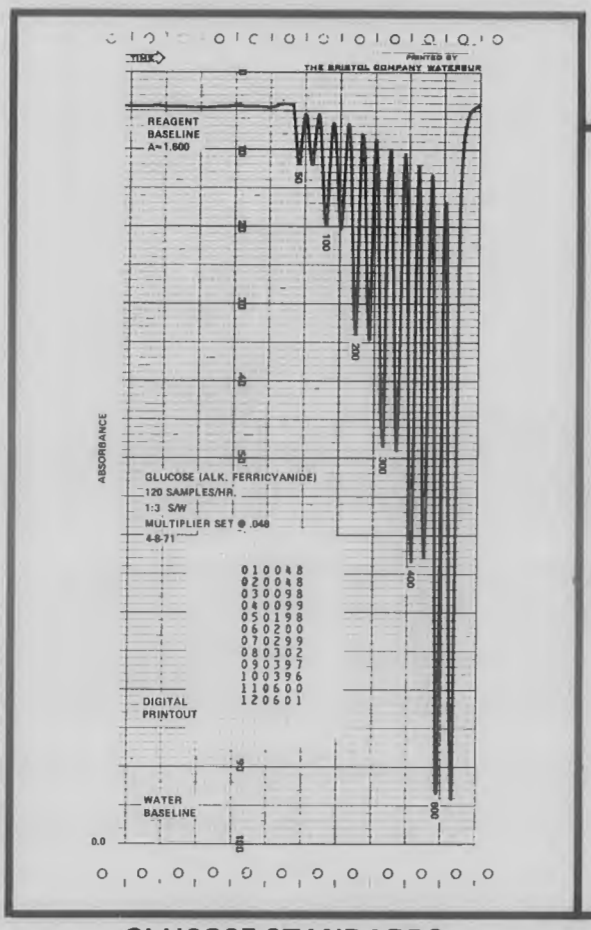
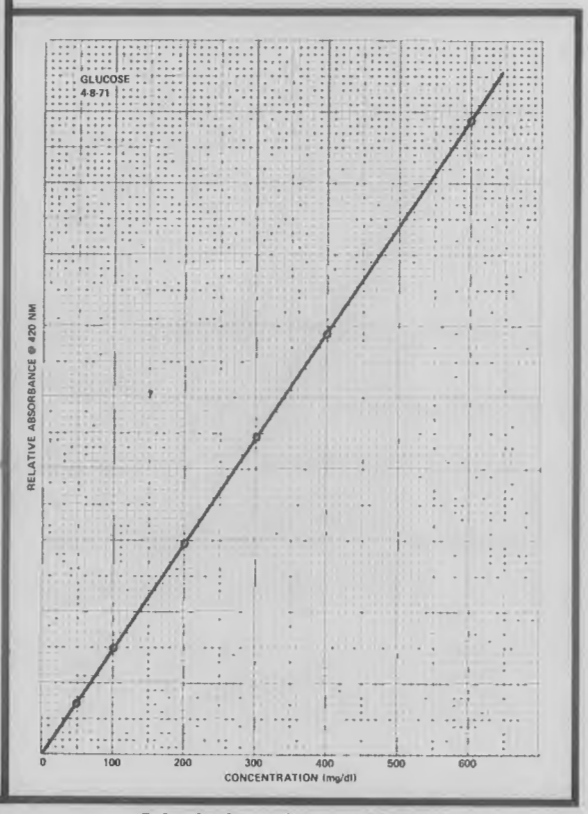


GLUCOSE STANDARDS



CALIBRATION CURVE

Neurology for Nurses. Edwin R. Bickerstaff, MD, FRCP. Second edition 1971. 156 pages. Black-and-white illustrations. English University Press. Price, £UK1.50.

As the title states this is a handbook of neurology for nurses, or other non-medical staff associated with neurology. From the medical technologist's point of view, it offers a source of reference for the meaning of the innumerable neurological diagnoses which may accompany laboratory requisitions. In this respect it offers a good concise description of each condition but the bias is towards clinical diagnosis and nursing care with very little reference to laboratory tests.

The structure and function of the nervous system is dealt with in 16 pages to a depth that would far from satisfy the TCA Medical Biology requirements, but does give one a slightly more than basic idea of the anatomy of the nervous system.

Chapter II headed 'The Language of Neurology' provides a very useful reference to words found throughout the text and obviates the necessity of consulting a medical dictionary, a chore which becomes frustrating in many other books of this type.

The appendix provides a table of blood analysis in neurological disease in which the units are ill-defined or not defined at all, i.e., serum potassium 12-18 mg (by calculation would approximate 3-5 mEq/litre). Serum acid phosphatase 1.5-4 mg means very little without a unit definition.

Despite these somewhat glaring omissions the book does provide a useful insight into the subject of neurology and it is refreshing to see that a physician of Mr Bickerstaff's standing can write with a sense of humour.

—D. A. H.

Artificial Cells. Thomas Ming Swi Chang. 1972. 207 pages. Published by Charles C. Thomas, Springfield, Illinois. Price $US16.

This is not a book of science fiction nor even a rarefied account of laboratory experiment, but a practical dissertation on the preparation and use of artificial cells.

The author, who is prominent in the field, makes use of a technique of micro-encapsulation. A form of this technique has received publicity of recent years by virtue of the commercial possibilities revealed in transporting liquids in a 'dry' form. In this particular instance, cells consisting of 'permanent' ultrathin cellulose nitrate polymer spheres containing a variety of biological or synthetic materials are prepared. These include cell homogenates, enzymes and detoxifying agents.

Some of the functions of living cells related to their physical characteristics can be copied. Thus an enormous surface area is presented for reactions to occur and the semi-permeable membrane permits selective reaction and rapid equilibration. Rejection or removal of such cells occurs rapidly unless protected by biologically compatible coatings.

Several different materials are used to prepare these cells, apart from cellulose polymers. Thus nylon, silicone and rubber have been used. The techniques of preparation are fully described. In interfacial precipitation the formation of the membrane depends on the lower solubility of a polymer dissolved in a water-miscible fluid. The micro capsules under appropriate circumstances precipitate and the supernatant is removed. Cells 5μ in diameter can be obtained. Interfacial polymerisation is used to prepare nylon cells. The cell content is incorporated in the aqueous phase in each case. Biological material such as cross-linked protein, lipid and polysaccharides have also been incorporated in the cell membrane.

There are chapters on the biological properties and activities. What are their uses? The potential is limited only by the imagination according to the author, and a wide range of experiments have been tried. For example, in enzyme therapy, models for replacement in congenital deficiences and for the suppression of substrate-dependent tumours have been set up. As a blood substitute free from antigens, fluorocarbon emulsified with a non-ionic detergent has been able to replace blood to the extent that the haematocrit can be reduced to 6 percent in experimental animals with survival and subsequent regeneration of the blood constituents.

In regard to practical application most success has been obtained with extra-corporeal shunts since cell survival is a problem *in vivo*. Thus blood, peritoneal fluid or kidney dialysate may be passed through a shunt with cells containing urease, exchange resin or activated char-

coal and having heparin bound to their surfaces. As an alternative to haemodialysis by the conventional artificial kidney, extra-corporeal shunts containing artificial cells provide a greatly increased surface area. It is stated that 10 ml of 20μ diameter cells would provide a surface area of 20,000 cm^2 which is greater than that provided by the conventional machines. The membrane thickness of the artificial cells is only one-tenth of the conventional membrane.

Clinical trials show that very efficient removal of creatinine, uric acid and quanidine can be achieved with encapsulated activated charcoal. Urea, water and electrolytes are not removed; although urease and ammonia trapping models have been successfully set up urea removal has not been perfected. Even so, selected patients unsuitable for conventional treatment have been successfully treated by this technique.

This is an absorbing book and should appeal to those interested in the development of new techniques in medicine and to those concerned with the repercussions in the laboratory.

—R. D. A.

Review of Medical Microbiology—Tenth edition (1972) by Ernest Jawetz, Joseph L. Melnick, and Edward A. Adelberg. Published by Lange Medical Publications. Price $8 from the supplier, N. M. Peryer Ltd.

This book requires no introduction as practically all bacteriologists will be familiar with previous editions. The new edition is slightly larger (518 pages) than the ninth edition (484 pages), but the general format is unchanged, and the chapter headings are identical.

Some illustrations have been changed, and those in chapter three 'The Major Groups of Bacteria' are more appropriate to the subject. 'Microbial Genetics' is better set out than previously. An account of some newer antibacterial chemotherapeutic agents is supplied in chapter ten, including Ethambutal and Rifampin. The chapters related to immunology have been up-dated. The first report of the International Committee of Viruses on the nomenclature of viruses is presented.

As in previous editions, systematic bacteriology is given a rather superficial coverage compared to virology. The latter appears to be disproportionately detailed, and the main expansion of the book has been in the chapters devoted to virology. Parasitology remains barely a sketch account.

This publication is directed mainly to the medical student, house officer, and practising physician. Medical students will use this book if it is an appropriate guide to the lectures they attend, but I don't think house officers will use it to any extent. Frankly, I believe it does not provide a well-balanced coverage of medical microbiology, despite the many excellent chapters it contains. There is inadequate reference to the interpretation of the common laboratory investigations, e.g., urine cultures, sputa, swabs, etc.

On the other hand, I consider this book should be among the select few kept on the shelves of a laboratory library. The general chapters on microbiology should be read by trainees preparing for the MLTB Part III examination. Those who possess the ninth edition can probably await the arrival of another edition before making a new purchase.

—P. H. P.

Elementary Microstudies of Human Tissues. James V. Bradley, MAT. 360 pages. 1972. Published by Charles C. Thomas. Price $US15.75.

This book was written to be used by student nurses, high schools, and training colleges. It is also suitable as a supplementary aid for NZCS students taking medical biology.

The introduction contains brief instructions on how to use the microscope, a very brief description of the preparation of histological slides and some hints on interpreting sections of tissue when seen through the microscope.

Six basic tissues are described (the author prefers to separate bone and cartilage and blood and lymph from the classification of connective tissue).

Chapters three to seventeen describe the various systems and organs. The location, basic function and major structural features of the organs are presented followed by a reasonably detailed description of the micro-structure of the organ. The book makes interesting reading but to be used as a textbook for medical biology a little more depth of description would be required. I think the book would be vastly improved if the diagrams were enlarged and made bolder. A few electron micrographs

would also help towards understanding more of the cells of some of the organs. No doubt the set of colour-photographed slides obtainable from the author (at an unstated price) would supplement the text, but I do not like the idea of having to buy a set of transparencies plus a projector and screen or pocket viewer in order to understand a book.

However, as stated earlier, I would recommend it as a supplementary aid for trainees.

—D. T.

Selected Abstracts

Contributors: D. G. Bolitho, Alison Buchanan, M. Jeannette Grey, J. Hannan, W. Stead, A. G. Wilson.

CHEMICAL PATHOLOGY

Blood Ammonia Levels and Their Significance in Diagnosis and Follow-up of Liver Disease. Englhardt, A. (1971), *Germ. Med.* **1**, 3, 113.

Ammonia levels in venous blood were measured in 50 normal subjects, 71 patients with liver cirrhosis and 21 other forms of liver disease. Venous ammonia content differed significantly in patients with chronic liver disease from that in normal persons but there was no significant correlation to the degree of coma. There was a progressive rise in ammonia levels in patients with liver cirrhosis and portal encephalopathy in the more severe stage. Mildly elevated values were sometimes found in hepatitis, congestion of the liver and alcoholic fatty liver. In liver cirrhosis with portocaval encephalopathy, only the mean blood ammonia levels were elevated while other laboratory tests were within normal limits. The blood ammonia levels did not in all instances correlate with the clinical picture: despite a fall in ammonia levels during treatment a change in the level of consciousness was not regularly observed in comatose patients.

—A. B.

Simplified Turbidimetric Assay for Lipase Activity. Shihabi, Z. and Bishop, C. (1971), *Clin. Chem.* **17**, 12, 1151.

Serum lipase is determined by following turbidity changes during two one-minute intervals after adding serum to an olive oil emulsion containing desoxycholate. The olive oil emulsion is simply prepared and is stable for a month under refrigeration. Our observations confirm the findings of other investigators, that increases in serum lipase activity during pancreatitis are more accentuated than increases in serum amylase activity. With the present fast and convenient method, serum lipase appears a better test for pancreatitis than is serum amylase.

—A. B.

Comparative Evaluation of Commercial Precipitating Antisera Against Human IgA. Phillips, D. J., Shore, S. L., Maddison, S. E., Gordon, D. S., Reimer, C. B. (1971), *J. Lab. Clin. Med.* **77**, 4, 639.

One experimental and 13 commercial antisera to human IgA were evaluated for antibody patency and specificity against a panel of 20 antigens with the use of reverse radial immunodiffusion in agar gel. Three commercial reagents were shown to be class specific, nine reacted inappropriately with human IgM; one did not precipitate with its homologous antigen IgA. A variety of human serum proteins used as soluble immuno absorbents was found in excess in most of the commercial antisera.

—A. B.

Serum Amylase Activity in Liver Disease. Bhutta, I. H. and Rahman, M. A. (1971), *Clin. Chem.* **12**, 12, 1147.

Serum amylase activity was measured in 15 normal persons and in 60 liver disease patients. Impairment of liver was assessed by serum bilirubin and thymol turbidity values. Most of the patients had serum amylase values that were well below the normal limits. Amylase activities were related to the degree of liver dysfunction and serum amylase decreased as the bilirubin and turbidity values increased.

—A. B.

Hyperlipemia: A Cause of Abdominal Pain. Hines, C. Jnr and Davis, W. D. Jnr (1972), *Sth. med. J., Birmingham*, **65**, 595.

Recurrent episodes of severe abdominal pain are known to be associated with Types I, IV and V hyperlipoproteinaemia. The very rare Type I may be diagnosed during childhood on the basis of creamy serum. The common Types IV and V are rarely detected before adulthood.

Three patients with recurrent abdominal pain of obscure aetiology are presented. *Case* 1 was noted by the laboratory to have 'very turbid' serum, while the serum of *Case* 2 was described as 'opalescent'. The serum of *Case* 3 was not described, but a triglyceride level of 1370 mg% was found.

Pancreatitis was demonstrated in one case: the mechanism of the development of abdominal pain in the other two cases is unknown.

—J. H.

Micro Estimation of Plasma Glucose Using Work-simplified Methodology. Georges, R. J. (1971), *S. Afr. J. med. Lab. Technol.*, **17**, 83.

This paper presents the results of a short study of any enzymic method and an *o*-toluidine method,

the methods investigated being selected on the basis of (1) small sample (20 μl), (2) final colorimetric reading and (3) ready adaptation to a system of work-simplification based on semi-automatic dispensing (Oxford dispenser) and diluting (Oxford diluter) equipment and a flow-through colorimeter with digital printout.

Using this equipment, rates of work output comparable to that of a single-channel autoanalyser could be maintained for short periods. Thus by either method a batch of 20 glucose estimations could be completed within 30 min.

It is felt that either method could prove satisfactory in routine use.

—J. H.

Spectrophotometric Standardization of Enzyme Assays. Martinek, R. G., Jacobs, S. L., and Hammer, F. E. (1972), *Clin. chim. Acta* **36**, 75.

In the absence of reliable standard reference material for enzyme assays of reasonable purity, stability, and cost, many enzyme units have been based on spectrophotometric absorbance units. This paper suggests the use of a liquid photometric standard, potassium dichromate solution, to guarantee 'absolute' photometric absorbance values eliminating differences between instruments and laboratories. The authors also propose a method to standardise instruments with band widths greater than 20 nm.

—A. G. W.

An Improved Colorimetric Procedure for Urine Oxalate. Hodgkinson, A. and Williams, Ann (1972), *Clin. chim. Acta* **35**, 127.

A method is described for the determination of oxalic acid in 0.5 ml of urine. Oxalic acid is coprecipitated with calcium sulphate, reduced to glycollic acid by boiling with dilute sulphuric acid and a zinc pellet, and estimated colorimetrically with chromotropic acid. The method is said to be more accurate and less time-consuming than existing chemical methods.

A. G. W.

Standardization of Mechanised Serum Alkaline Phosphatase Determinations. Horn, D. B. (1972), *Clin. chim. Acta* **37**, 43.

The author describes the use of a modified King and Kind method, and the use of phenol standards in alkaline solution to eliminate absorption of phenol by the sample pump tube. Because consistent and reproducible results were obtained a correction factor commonly used to give comparability with the manual method was eliminated.

—A. G. W.

Serum Albumin Estimation: Modification of the Bromcresol Green Method. McPherson, I. G. and Everard, D. W. (1972), *Clin. chim. Acta* **37**, 117.

Difficulties encountered during the estimation of serum albumin with a buffered bromcresol green reagent were investigated, and a modified method to overcome them is detailed and evaluated. The authors have used a glycine-HCl buffer to eliminate precipitation and alcohol as the dye solvent. Improved sensitivity with these reagents is claimed.

—A. G. W.

Problems in the Measurement of Iron Binding Capacity in Serum. Williams, H. L. and Conrad, M. E. (192), *Clin. chim. Acta* **37**, 131.

The magnesium carbonate method for measurement of TIBC and UIBC was studied to disclose causes of error and improve techniques. Failure to regulate the pH of reaction mixtures at all stages of the procedure and inadequate amounts of protein in the specimen each caused a significant error in measurement of both the 'total' and 'unsaturated' iron binding capacities. The use of undiluted serum, regulation of the pH of the iron saturating solutions and the addition of sodium barbital buffer to serum before the addition of iron saturating solution seemed to improve results.

—A. G. W.

A Rapid Photometric Micro Method for Serum Lipase Determination. Lippe, U., Stevanato, G., and Guidi, G. (1972), *Clin. chim. Acta* **37**, 199.

A new micro method for the determination of serum lipase is described. 0.1 ml of serum is incubated for 30 minutes at 40° in a substrate consisting of olive oil and desoxycholic acid. The pH of the substrate is 8.5. The hydrolised fatty acids are measured colorimetrically by a slight modification of Duncombe's method.

A. G. W.

CYTOLOGY

Improved Prognosis for Cervical Cancers Due to Comprehensive Screening. Macgregor, J. E., Fraser, M. E., and Mann, E. M. F. (1972), *Acta Cytologica* **16**, 14.

After 10 years of comprehensive screening in Aberdeen, 95% of the women aged 25-56 years have had cervical smears taken.

The structure of the population is unaltered and there is now a marked fall in the incidence of cases of overt clinical cancer. The fall is most marked in women under 60 years, whereas in 1950 the majority of cases occurred in women under 60 years of age, the majority of cases now occurring are in older women.

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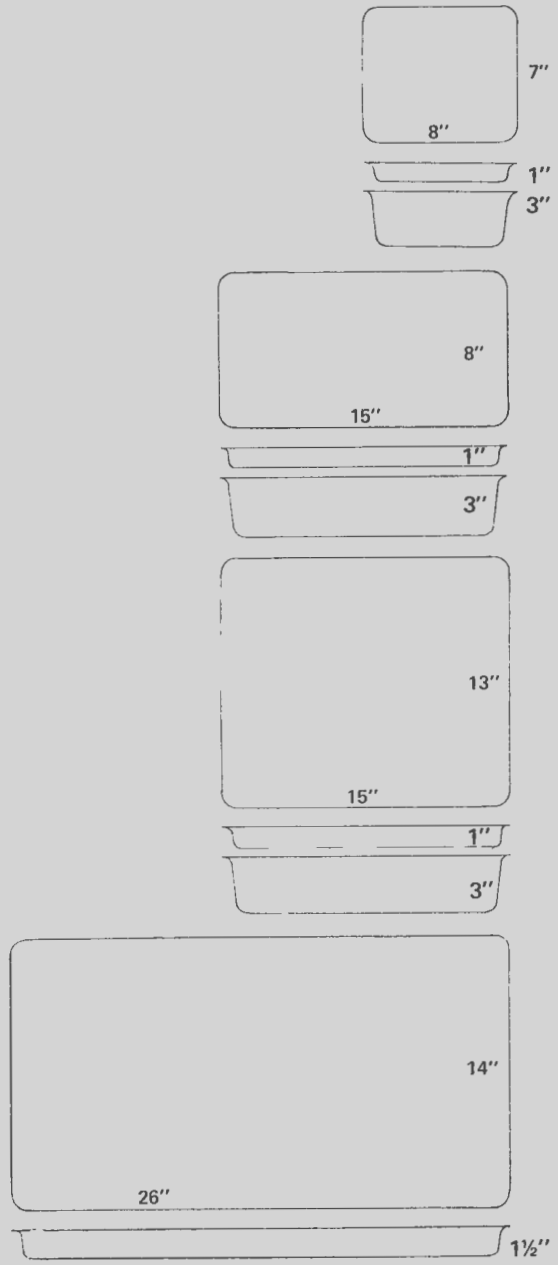

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If the micro-invasive (occult invasive) cases are included, the 5-year survival rate has increased from 55% in 1942 for Stage I cancer, to 82% in 1964 for Stage I and IA.

—W. S.

Vaginal Cytology and Amnioscopy in Prolonged Pregnancies. Nyklicek, O. (1972), *Acta Cytologica* **16**, 48.

Vaginal smears were examined and amnioscopy performed in 100 women with prolonged pregnancies. Results of vaginal cytology and amnioscopy were compared shortly before delivery. The regressive pattern in cytology together with the finding of green amniotic fluid in amnioscopy represents an indication for the induction of delivery.

Vaginal cytology as a method for follow-up of hormonal function of the placenta and amnioscopy as a procedure diagnosing acute danger to the foetus are complimentary methods.

They extend information regarding the foetus not only in prolonged pregnancy but also in other cases. Both procedures are simple and can be performed in all hospitals.

—W. S.

Comparison Between Diagnostic Results Obtained by Transthoracic Needle Biopsy and by Sputum Cytology. Dahlgreen, S. E. and Lind, B. (1972), *Acta Cytologica* **16**, 53.

Diagnostic results were compared for a series of 125 patients from all of whom transthoracic needle biopsy material and at least one satisfactory sputum specimen had been obtained. Of these patients 101 had malignant tumours in their lungs, 93% of which were diagnosed by aspiration biopsy and 64% by sputum cytology. Only four benign tumours were represented. None of these could be detected by sputum cytology, but two were correctly identified and a bronchial cyst was suspected by means of aspiration cytology.

Nineteen patients had various inflammatory lesions. In diagnosing these aspiration cytology methods proved superior to sputum cytology.

—W. S.

Calcified (Psammoma) Bodies in Alveolar Cell Carcinoma of the Lung. Gupta, R. K. and Verma, K. (1972), *Acta Cytologica* **16**, 59.

The presence of round, concentrically laminated calcified bodies with tinctorial features of psammoma bodies has been described in three cases of bronchioalveolar carcinoma of the lung. Psammoma bodies were present in the sputum in one case and the pleural fluid in the other two cases.

These cases are being documented because of their extreme variety.

The diagnostic significance of psammoma bodies whenever found in sputum and body cavity fluids has been discussed.

—W. S.

Plant Cells Resembling Tumour Cells in Routine Cytology. Avrin, Erna, Eugena, Margaret, Schwarz, Ruth, and Sobel, H. (1972), *A. J. C. Path.* **57**, 303.

Cells with large, dark, clumped nucleus-like bodies are occasionally observed in routine vaginal cytologic preparations.

They resemble tumour cells but often on closer scrutiny, are found to have thick cell walls not unlike those of vegetable cells, and are identical to structures found in gum guar and lubricating jelly which contains gum guar. The composition of every product should be marked clearly on the container.

Lubricating jellies which contain cellular material should not be used for cytologic preparations.

– W. S.

HAEMATOLOGY

A Rapid Method of Electrophoresis of Erythrocyte Glucose-6-phosphate Dehydrogenase on Cellulose Acetate Plates. Ellis, N. and Alperin, J. B. (1972), *Amer. J. clin. Path.* **57**, 534.

The separation of variants of G-6-P D in haemolysates is achieved in 15 minutes by electrophoresis at room temperature on hypergel cellulose acetate (Titan III) supported on a clear plastic base; Tris-EDTA brate buffer at pH 9.1 is used. The authors found it unnecessary to wash erythrocytes before lysis and used only water and 4.5% sodium chloride in the preparation of the haemolysate. Staining was only for 15 seconds. There were eight samples per acetate plate (6 cm by 7.8 cm). The power supply was set at 300 v with milliamps not in excess of seven. This method will screen 100 haemolysates in a working day of eight hours.

—M. J. G.

Hepatitis-associated Antigen: Detection by Antibody-sensitized Latex Particles. Fritz, R. B. and Rivers, Shirley L. (1972), *J. Immun.* **108**, 108.

This report describes the development of an antibody-sensitised latex particle agglutination assay. This method was found to be somewhat more sensitive than complement fixation and it may be done rapidly with equipment available in serology laboratories. By use of agglutination inhibition, antibodies to hepatitis-associated antigen may be readily detected.

—J. H.

The Polychromatophilic Erythrocyte. Perrotta, A. L. and Finch, C. A. (1972), *Amer. J. clin. Path.* **57**, 471.

An important paper showing that the polychromatophilic erythrocytes seen in blood films equate with

the very immature reticulocytes and not with all reticulocytes. Thus normally polychromatophilic erythrocytes represent 5% or less of the circulating reticulocyte population, and have a mean diameter 27% greater than mature erythrocytes. The reticulocytes in this study were graded into four groups or stages of maturation by the amount of reticulum (RNA) in the cell.

Polychromatophilic erythrocytes in the peripheral blood film provide a simple means of identifying erythropoietin stimulation of the erythroid marrow. With moderate hypoxia or anaemia, they increase to between 10 and 20%; and to over 20% with severe anaemia.

—M. J. G.

Disseminated Intravascular Coagulation in Diabetes Mellitus, with Reference to the Role of Increased Platelet Aggregation. Kwaan, H. C., Colwell, J. A., and Suwanwela, N. (1972), *Diabetes* 21, 108.

This paper describes the clinical and postmortem findings of a patient with diabetes mellitus, who died from disseminated intravascular coagulation (DIC) and whose plasma, which was grossly lactescent, was found to greatly enhance the adenosine diphosphate induced aggregation of normal platelets.

It is postulated that the strong influence of the patient's plasma on platelet aggregation was the initial event leading to the DIC.

The marked lactescence was deemed responsible for a falsely high result in a haemoglobin determination.

—J. H.

DNase Enzyme in Haematological Diagnostics. Berzy I. (1971), *Haematolog.* 5, 431.

Blast cells were studied by treating bone marrow and peripheral blood smears with a preparation of DNase and then staining according to Pappenheim. It was found that the DNase hydrolysed the nuclei of myeloid cells, while the nuclei of lymphoid cells and erythroblasts were not affected. The nuclei of monocytes and reticular elements were affected only to a slight extent. From the 200 smears studied, the test was found useful in the differentiation of blast cells, evaluated in correlation with results of the common enzyme cytochemical methods. This paper includes 12 good coloured photomicrographs illustrating the use of DNase

—M. J. G.

Hereditary Nonspherocytic Haemolysis with Erythrocyte Phosphofructokinase Deficiency. Waterbury, L. and Frenkel, E. P. (1972), *Blood* 39, 415.

A case of a young man with only 60% of normal erythrocyte phosphofructokinase activity, no anaemia but a reticulocyte count of 6%-8%

Full laboratory investigations are described. The 48-hour autohaemolysis test revealed a slight increase in haemolysis that was corrected with glucose. Erythrocyte cation concentrations were normal. The serum bilirubin was 2.6 mg/100 ml; bone marrow revealed erythroid hyperplasia. This paper includes full details of the enzyme studies. Erythrocyte ATP levels were depressed.

—M. J. G.

Nuclear Sex Determination in Infertile Males. Sen, S. C. and Thombre, D. P. (1971), *Indian J. med. Res.* 59, 1761.

Nuclear sexing has aroused keen interest to detect sex chromosomal abnormalities in general as well as in selected cases. The finding that a phenotypic male possessing a sex chromatin body may be genetically female and may not be fertile gave an impetus to screening subfertile and infertile males through nuclear sexing. The chromosomal constitution of chromatin-positive males resembles that of genetic females in having 2 X chromosomes in their body cells, but in addition they invariably have a Y chromosome, as in males. In the general population, about 2 per 1,000 males are found to be chromatin-positive although an Australian study reported 6 per thousand. The higher incidence of such cases in subfertile males has been determined by a number of workers.

In the present study, blood films on slides were Leishman-stained and at least 1,000 neutrophils examined for the presence of sex chromatin. The frequency of chromatin-positive males in highly subfertile cases attending an infertility clinic was found to be low (6.5%) compared to surveys carried out by other workers.

—J. H.

MICROBIOLOGY

Morphological and Biochemical Studies of 27 Strains Belonging to the Genus Aeromonas Isolated from Clinical Sources. Zajc-Satler, Jadranka (1972), *J. med. Microbiol.* 5, 263.

Members of the genus *Aeromonas,* previously not considered pathogenic to man, have in recent years been increasingly incriminated as causes of clinical disease. This report gives the result of a study of morphological and biochemical characters of 27 strains of aeromonads isolated from human clinical sources. The organisms were identified as 27 strains of *Aeromonas hydrophila* biotype I, one strain of *Aeromonas hydrophila* biotype 2, one strain of *Aeromonas punctata* and four strains of *Aeromonas punctata* sub species *caviae.* 24 of the strains were isolated from patients with enterocolitis.

—D. G. B.

Urinary Tract Infection and Oral Penicillin G. Holbert, J. (1972), *J. clin. Path.* 25, 73-75.

Measurement of the urinary levels of penicillin after administration of oral penicillin G are examined

in this paper. It was found that urinary tract levels of penicillins efficient to destroy many Gram negative urinary tract pathogens are attainable by dosages of penicillin administered by this route and that, therefore, it is a rational way to treat urinary tract infections due to Gram negative bacilli. Three types of oral penicillin G preparations were examined in a very limited number of 6 patients.

—D. G. B.

Erwinia Species: An Emerging Human Pathogen. Bottone, E. and Schneierson, S. S. (1972), *Amer. J. clin. Path.* 57, 400-405.

The plant pathogen of the genus *Erwinia* have recently been appearing in human infections from a variety of sites. This paper discusses identification criteria and cultural methods for this new group of pathogens.

—D. G. B.

Primary Amoebic Meningoencephalitis in California. Hecht, R. H., Cohen, A. H., Stoner, J., and Irwin, C. (1972), *Calif. Med.* 117 (1), 69.

Primary amoebic meningoencephalitis, to be distinguished from infections caused by *Entamoeba histolytica,* is a newly recognised disease entity. It is an overwhelming, rapidly fatal disease which affects a previously healthy child or young adult with a history of recent swimming in fresh-water lakes, brackish water or pools of diverted river water. The causative organism is usually a ubiquitous, free-living soil amoeba of the Naegleriidae family (*Naegleria* sp).

Epidemiologic observations of this disorder unassociated with swimming should be emphasised. Primary amoebic meningoencephalitis has occurred in a patient with advanced alcoholic liver disease who had dental extractions. Three cases have occurred in children playing in a warm, muddy puddle. Furthermore, the isolation of *Hartmanella* spp from normal throats has been noted.

The diagnosis is invariably confirmed by the findings of motile trophozoites in a cerebrospinal fluid wet mount. This should be a routine examination in any case of meningitis.

—J. H.

Allergy of the Lower Urinary Tract. Powell, N. B., Powell, Elizabeth B., Thomas, O. C., Queng, J. T., and McGovern, J. P. (1972), *J. Urol.* 107, 631.

The urinary tract contains the same tissues (mucosa, many blood vessels and much smooth muscle) that are affected by immunological reactions in other parts of the body and the authors feel that urinary tract allergy is seldom recognised. During an 11-year period more than 900 urologic patients refractory to conventional treatment were found by the authors to be allergic.

Several reports have suggested that the presence of eosinophils in the urine would be important in diagnosis but the authors found that this was not a reliable diagnostic test owing to the rapid disintegration of eosinophils in urine.

Recent studies by other workers have indicated high synthesis of immunoglobulin E in the urinary tract. This immunoglobulin contains the majority of the reaginic antibodies that are responsible for the allergic reaction in the respiratory and gastrointestinal mucosae, in nasal polyps and secretions of atopic patients and in sputa of asthmatic patients.

—J. H.

Problems in the Use of the In Vitro Toxigenicity Test for *Corynebacterium diphtheriae*. Bickham, S. T. and Jones, W. L. (1962), *Amer. J. clin. Path.* 57, 244-246.

This paper discusses difficulties experienced in the Elek test for toxigenicity in diphtheria, describes a modification of the base media, and dilutions of the antitoxin designed to overcome the difficulties described in the paper.

—D. G. B.

Trimethoprim-resistant Coliforms. Lacey, R. W., Bruten, D. M., Gillespie, W. A., and Lewis, E. L. (1972), *Lancet* 1, 409.

This paper reports a 2.5% incidence of resistance to trimethoprim of coliform bacilli isolated from patients in a large city in the West of England. Five of the 18 resistant organisms isolated were from patients who had previously been treated with trimethoprim/sulphamethoxazole.

The authors warn that resistance to this antibiotic appears to be increasing, and that in view of its clinical usefulness it should be reserved for those infections which particularly warrant it and not used indiscriminately.

—D. G. B.

Effect of Gentamycin on Growth of Yeasts, Yeast-like Organisms and *Aspergillus fumigatus*. Dolan, C. T. (1972), *Amer. J. clin. Path* 57, 30-32.

This paper extends a previous study by the author into the optimum levels of antibiotics in media for the isolation of fungi and Nocardia from man. The conclusion reached is that chloramphenicol at 16 μg/ml and gentamycin at 5 μg/ml would not inhibit fungi but would inhibit most bacterial contaminants. An added advantage is that both of these antibiotics can be autoclaved without loss of potency.

—D. G. B.

Cary-Blair, a Transport Medium for *Vibrio parahaemolyticus*. Neumann, D. A., Benenson, M. W., Hubster, E., and Nguyen Thi Nhu Tuan (1972), *Amer. J. clin. Path.* 57, 33-34.

This paper discusses the effectiveness of the non-nutritious transport medium of Cary-Blair in preserving *Vibrio parahaemolyticus* or rectal swabs. It was found that the organisms could be recovered after 35 days at room temperature. The medium has previously been demonstrated to preserve *Salmonella* and *Shigella* species for up to 30 days at room temperature. This appears to be a most useful faecal transport medium. *Vibrio parahaemolyticus* is of

increasing importance in many areas of the world as a cause of diarrhoea due to the eating of contaminated sea-food.

—D. G. B.

The Isolation of an X Dependent Strain of Haemophilus from Otitis Media Identified as *H. haemoglobinophilus (Canis)*. Frazer, J. and Rogers, K. B. (1972), *J. clin. Path.* 25, 179-180.

This paper describes a case of *Haemophilus haemoglobinophilus* in an infant with severe hypogammaglobulinaemia. The cultural characteristics of the organism are described. The case is interesting in that there appears to be no previous record of a *Haemophilus canis* or *Haemophilus haemoglobinophilus* infection in man. No close association between the patient and a dog could be discovered in this case.

—D. G. B.

Quality Control in Bacteriology Preliminary Trials. Stokes, E. J. and Whitby, J. L. (1971), *J. clin. Path.* 24, 790-797.

A quality control system in bacteriology is discussed. Twenty-five laboratories took part, a total of six sets of quality control material were sent out as simulated specimens but known to the recipients as quality control material. Each trial batch specimens consist of six specimens: three faeces, two swabs, one urine.

The authors discussed the problem associated with the manufacture of simulated specimens and give a full discussion of the results of the findings from the various laboratories. They comment unfavourably on the speed of reporting of some recipients, and on the standard of antibiotic sensitivity test results.

This paper is further evidence that interest has at last been aroused in quality control in microbiology.

D. G. B.

Sensitivity of *Pseudomonas pseudomallei* of Trimethoprim and Sulphamethoxazole in Vitro. Bassett, D. C. J. (1971), *J. clin. Path.* 24, 798-800.

This short paper suggests that trimethoprim/sulphamethoxazole used as a long-term treatment could be a useful alternative to antibiotic therapy in the treatment of this condition.

D. G. B.

Bacteriocins from *Neisseria gonorrhoea* and their Possible Role in Epidemiological Studies. Flynn, J. and McEntegart, M. G. (1972), *J. clin. Path.* 25, 60-61.

This short paper describes experiments in bacteriocin typing of *Neisseria gonorrhoea*. The authors claim that at present with six indicator strains 75% of isolates can be characterised. They suggest that with further work it may well be possible to 'gonocin' type *Neisseria gonorrhoea*.

—D. G. B.

Coagulase Negative Strains of *Staphylococcus pyogenes*. Bayston, R. (1972), *J. clin. Path.* 25, 62-64.

This paper describes two undoubted strains of *Staphylococcus pyogenes* which failed to produce coagulase despite intensive efforts to demonstrate this. Both organisms were confirmed as being *Staphylococcus pyogenes* and phage typable by an independent laboratory and both were D-Nase positive.

The authors conclude that the coagulase test alone is unsatisfactory for the identification of *Staph. pyogenes* and recommend the use of D-Nase test in addition.

—D. G. B.

The Effect of Passage and Iron on the Virulence of *Pseudomonas aeruginosa*. Forsberg, C. M. and Bullen, J. J. (1972), *J. clin. Path.* 25, 65-68.

This paper discusses the fact that iron compounds greatly enhance the virulence of *Pseudomonas aeruginosa* and in experimental studies virulence is much enhanced if the serum transferin of the host is saturated with iron.

The authors stress the dangers of passage from patient to patient in enhancing the seriousness of an outbreak of *Pseudomonas aeruginosa* infection and suggest that transferin, normally unsaturated with iron may play an important part in resistance to infection with this organism.

—D. G. B.

An Evaluation of Three Methods of Typing Organisms of the Genus Proteus. Tracey, O. and Thompson, E. J. (1972), *J. clin. Path.* 25, 69-72.

Comparison of three methods of Proteus typing presented. Biochemical typing, bacteriocine typing and typing by the Diene's phenomena.

The authors concluded that the Diene's phenomena is the most useful method of differentiating Proteus strains. There is a short discussion of their relationship of the Diene's phenomenon to bacteriophage production in Proteus organisms.

D. G. B.

Cetrimide-Nalidixic Acid Agar as a Selective Medium for *Pseudomonas aeruginosa*. Lily, H. A. and Lowbury, E. J. L. (1972), *J. med. Microbiol.* 5, 151.

This paper describes a comparative trial of 0.03% cetrimide agar (Brown and Lowbury) with a medium containing 0.02% cetrimide and 15 μg/ml of nalidixic acid as suggested by Gata and Enomata (1970). This latter medium has been claimed to suppress many of the Providence and Klebsiella strains which will grow on cetrimide medium on its own. The trial demonstrated that the medium incorporating nalidixic acid yielded a large number of *Pseudomonas aeruginosa* isolates and a lower growth incidence of contaminating organisms.

—D. G. B.

The Lethal Effect of Typtone-Soya Broth. Waterworth, P. M., *J. clin. Path.* 25, 227.

The failure of several Vibrio and *Streptococcus pneumoniae* isolates to grow on sub-culture after overnight incubation in tryptone soya broth was investigated. This failure to grow was found to be due to a pH drop due to the fermentation of glucose in the medium. It is suggested that both tryptone soya broth and brain heart infusion broth with glucose are unsuitable for use as a blood culture medium.

—D. G. B.

Quality Control Testing With the Disk Antibiotic Susceptibility Test of Bauer-Kirby-Sherris-Turck. Blazevic, D. J., Koepcke, M. H., and Matsen, J. M. (1972), *Amer. J. clin. Path.* 57, 592.

A method of quality controlling the disk antibotic susceptibility testing method of Kirby and Bauer is described. The methods advocated are in general, similar to those which have featured commonly in the literature over the past two to three years. An interesting variation is the suggestion of the use of quality control charts to plot zone size readings plotted to plus to minus two standard deviations in the same manner as biochemical tests. One comment of the authors should be challenged; that it is not possible to perform a quality control check on the disk actually used on a given organism. While this is true of the Kirby-Bauer technique it is not true of the Stoke's method. This is a valuable paper and should be read by all interested in quality control in microbiology.

D. G. B.

Antibiotic Sensitivity Testing. Survey Undertaken in September 1970 in the United Kingdom. Castle, A. R. and Elstub, J. (1971), *J. clin. Path.* 24, 773-778.

This paper describes a survey of antibiotic sensitivity testing carried out two years ago in the United Kingdom. The paper describes the purpose of the survey, the types of hospital responding, the techniques and test materials, the variations in the type of sensitivity test medium, inoculum, spreading of the inoculum, disk content and the range of drugs used. It also discusses the number of drugs used and the choice of drugs.

Some very surprising information emerges from this survey of 330 hospitals. It is surprising to see that in spite of much published work, some hospitals test as many as 17 antibiotics on one single test plate. It is equally surprising to find that only 46% of all hospitals control their sensitivity tests with one or more standard organisms. The paper offers a comprehensive survey of current practice in the testing of organisms to antibiotics in the United Kingdom. It is extremely detailed with a comprehensive range of tables giving a great deal of information.

The authors feel that the range of methods is disquieting and that the adoption of a standard procedure for the performance of sensitivity tests would be desirable. This paper should be required reading in every laboratory dealing with antibiotic sensitivity testing.

D. G. B.

New Selective Media for Isolation of *Streptococcus haemolyticus*. Hasuzo Nakamizo and Maroto Sato (1972), *Amer. J. clin. Path.* 57, 228-235.

Two rather complex selective media for the isolation of *Streptococcus haemolyticus* are described —a liquid and agar medium. The liquid medium which the authors term enriched urease broth is a three-solution mixture using sodium azide and DL-camphor as selective agents. The agar medium, a two-part medium which does not require the addition of blood and uses a mixture of sodium azide and crystal violet as selective agents. Both media use urea as a growth stimulant. The media appear to be rather complex to prepare and the yeast extract powder described would probably not be available outside Japan. The authors claim that these new media gave a very substantial increase in the isolation rate of beta haemolytic streptococci from throat swabs; 47.2% with the new media compared to 23.6% of 1,225 throat swabs using anaerobic blood agar. They also claim that 11 throat swabs collected from patients after antibiotic therapy had been commenced could only be isolated by the new culture method. The media may merit further examination, but the difficulties of preparation will militate against its general adoption.

—D. G. B.

The Rectal Culture as a Test of Cure of Gonorrhoea in the Female. Schroeter, A. L. and Reynolds, Gladys (1972), *J. infect. Dis.* 125, 499.

Culturing the anal canal in addition to the cervix significantly increases the number of cases diagnosed. In this study, the rectal and cervical sites were compared as a test of cure in 908 patients who had gonorrhoea. After treatment, positive cervical or rectal cultures (or cultures of both sites) were found in 10.6% of the patients. Thirty percent of the therapeutic failures would have been missed if only the cervical site had been tested. Thayer-Martin medium was used.

—J. H.

A Study of Antibiotic Sensitivity Testing with Proposals for Simple Uniform Methods. Carrod, L. P. and Waterworth, P. M. (1971), *J. clin. Path.* 24, 779-789.

This paper deals with the findings of the survey of Castle and Elstub. It comments on the methods used by respondents to Castle and Elstub's questionnaire and offers a simple scheme for sensitivity testing. This again is an important paper which should be required reading in all bacteriology laboratories carrying out sensitivity tests.

—D. G. B.

What's New?

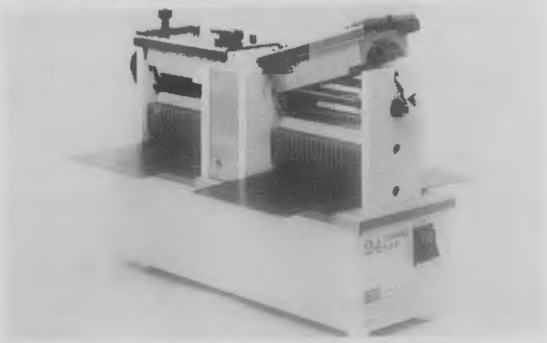
24-CHANNEL PUMP

Breda, The Netherlands.—A new 24-channel precision tubing pump has been developed by Cenco Instrumenten Mij. N.V. for use in automating chemical analyses.

Two roller cages for 12 pumping tubes each move synchronously at 10rpm, driven by a high-power, synchronous motor. The precision stainless steel bars in the roller cages are individually fitted in ball bearings and compress the tubing against a counter plate with micron accuracy. The pressure plate is spring fitted to protect the tubing and pumping lines from overpressure while the special curve of these plates diminishes pulsations as inherent in tubing pumps.

The 24 tubes are split over two roller cages driven by the same motor which allows replacing or priming one-half of the tubes while the other half works. Tubing sets are rapidly exchangeable in removable pump tubing racks, which can be set at five different distances to allow for tubing stretch and the use of different tubing.

Discharge liquid pressure is guaranteed at 2½atm, the pump is dripwater protected and provided with 3-wire line cord and fuse.



HISTOCON—A NEW SWEDISH INVENTION A TRANSPORT SOLUTION FOR USE IN HISTOCHEMICAL WORK

Bethlehem Trading Ltd, Box 12035, 402 41 Goteborg 12/Sweden.—The common fixative-solutions used in histological and histo-pathological routine are known to possess considerable enzyme-inhibiting and protein-extracting capacities. A histochemical or enzymehistochemical evaluation of a tissue biopsy has hitherto necessitated an almost immediate transfer of the biopsy from the surgical operation room to a cryostat without fixation fluid of any kind.

This means that histochemical investigations have been difficult to perform in case of transport problems. The tissue specimen has been quickly attacked by bacteria or destroyed by autolysis. The use of fixation fluid such as formalin on the other side has caused heavy leakage of proteins and other components, for example, polysaccharides and lipids, out into the fluid resulting in a more or less complete failure of histochemical methods.

To sum up, the properties of Histocon are the following ones:

(a) Bacteriolytic effect.

(b) Inhibition of autolysis.

(c) Reduced leakage of protein and other cellular components.

(d) Preservation of catalytically active enzymes (at +4°C within 48 hours after excision of tissue).

Routine process techniques for light microscopy are immediately applicable on tissue biopsies transported in Histocon as well as techniques for electron microscopy.

Histocon is used in the following manner:

1. The Histocon-solution is stored in refrigerator (±0°-4°C).
3. The tissue is put into 50-100 ml cool Histocon immediately after the excision.
3. The tube containing the tissue in the Histocon-solution is sent to the laboratory in a thermos flask with ice cubes from a refrigerator.
4. The temperature must not surpass +4°C at any moment during the transport.
5. The tissue must arrive to the laboratory not later than 48 hours after the excision if the aim is enzymehistochemistry. For other kinds of morphological analysis the times are less strict.

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VARIAN PUBLISHES BASIC LIQUID CHROMATOGRAPHY TEXT

Palo Alto, California.—Varian Instrument Division has published a 270-page text: Basic Liquid Chromatography.

The book presents the basic aspects of modern high-performance LC in a simple and understandable manner. Practical applications are emphasised, and references to more complete and rigorous treatments are provided.

Chemists interested in applying LC to analytical problems and technicians utilising LC but needing to know more about the technique, should find this book helpful. The text is intended to fill the gap between instruction manuals provided with commercial instruments and advanced treatises written by leaders in the LC field.

Copies are $US7.50, and may be obtained by writing Varian Instrument Division, 2700 Mitchell Drive, Walnut Creek, California 94597.

PRETRON ELECTRONIC COLORIMETER 3000B

This instrument is probably unknown in most New Zealand laboratories. It is manufactured by Pretron Electronics Pty Ltd, Western Australia. Some of its features which appeal to laboratories purchasing automated equipment are . . .

Wide spectral range without need for detector change, no aperture adjustment, high level analog

output peak detector and compatibility for use with automated data-gathering system.

The instrument uses a single monochromatic filter for each wavelength. Any difference in transmission characteristics of filters is automatically compensated for by the colorimeter light source. The electronics compartment is of modular construction utilising 'plug in' printed circuit boards. The electronic circuitry makes full use of the latest semiconductor integrated circuit devices.

The instrument is competitively priced at $1,282 with filters at $30. Optional extras include . . .

1. Automatic peak detector cards which enable a peak value to be stored. After interrogation via the digital address the peak is transferred and peak seeking continues.

2. Lineariser card: The normal output from the basic colorimeter follows Beer's Law. By replacing the linear amplifier card with the lineariser card linear absorbance characteristics are generated, giving improved resolution at high concentrations.

3. Computer interface cards can also be supplied to meet customer requirements.

The output range is from 0-10 volts thus the instrument can be used with any potentiometer recorder. We at Palmerston North are currently using a Rikadenki Model B261 and we are very pleased with the results.

Further information is available from Pretron Electronics Pty Ltd, 19 King Edward Road, Osborne Park, Western Australia. Currently there are no New Zealand agents, representation will be announced shortly.

NEW MATERIAL PREVENTS BODY ODOUR

The many problems associated with the unpleasant odour of perspiration can be solved, according to the Europa Chemical Company (55 Whitfield Street, London, England W1A 2BX), a Reed International company, with their new compound called Infrafresh, which completely stops the growth of certain bacteria and fungi.

FIG. 1.—Typical laboratory test: an untreated sample of fabric is quickly covered by fungal growth in an agar culture dish.

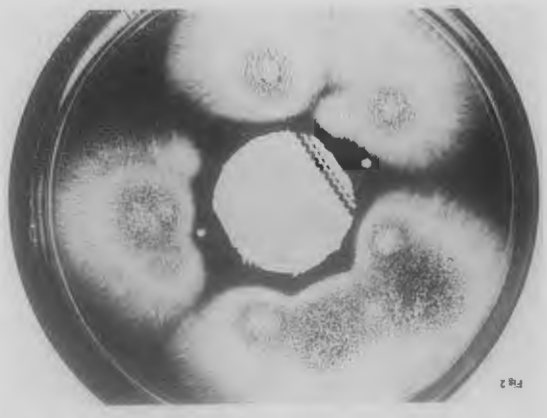

FIG. 2.—An Infrafresh-treated sample of the same fabric as in Fig. 1 remains unaffected and inhibits fungal growth around it.

Infrafresh is a liquid which can be applied to most materials, including textiles of natural and synthetic fibres, leather, plastics, rubbers, paper and others. Independent laboratory tests have proved that it is virtually non-toxic on treated products and remains active almost indefinitely—even after 400 launderings of roller towelling bacteriostatic effectiveness is still 100 percent.

Perspiration itself is odourless, but when it is decomposed by naturally occurring bacteria, an unpleasant smell results. Most anti-perspirants and deodorants inhibit the sweat glands, but only where they are applied. The body then increases its perspiration in other areas, thus shifting the problem without solving it. Some other preparations, which kill the bacteria, can be highly toxic and may leach out quickly in laundering and drycleaning. Infrafresh simply stops bacteria from multiplying and thus prevents decomposition, smell and degradation of the

fabric, yet normal body functions are not interfered with.

Extensive wearer tests already carried out by some of the leading manufacturers of textiles, hosiery, underwear, clothing, shoes, carpeting, furnishing, drapery and other consumer goods, have shown that Infrafresh can be incorporated without difficulty in existing manufacturing processes, remains fully effective throughout the natural life of the product and in no way affects handle, feel, colour and other characteristics.

Because Infrafresh inhibits fungal as well as bacterial growth, it has proved effective against microorganisms which cause such common ailments as athlete's foot, diaper rash, some forms of furunculosis, vaginal discharge and others. Considerable interest is therefore also be shown in the product for hygiene reasons.

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

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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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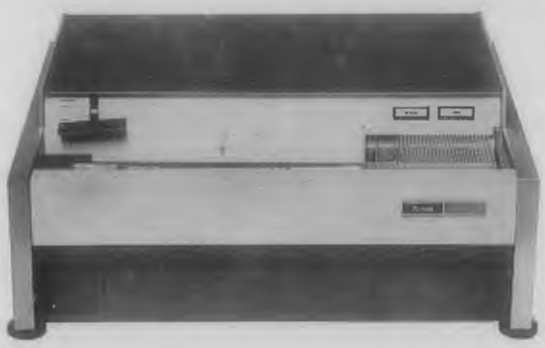

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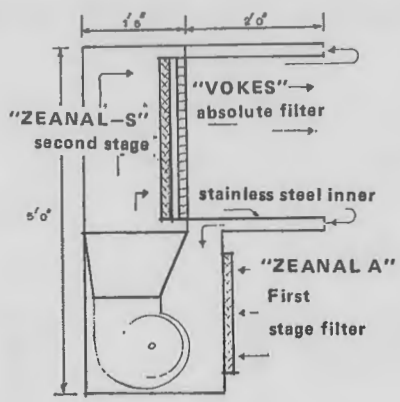

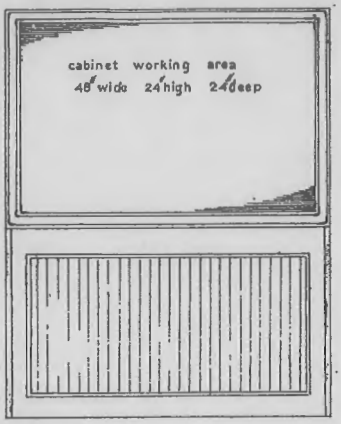

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